THE Rh FACTOR

In the Clinic and the Laboratory

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PREFACE

The Rh Factor

THE discovery of the presence of the Rh agglutinogen in the red blood cell must rank as one of the important events of a medical era already surfeited with important and exciting discoveries. It is good that Karl Landsteiner, the discoverer of the original blood groups, should have been the co-discoverer of the new blood factor. Here was a man so single minded in his devotion to the immunological aspects of the blood that for a period of at least 40 years he worked almost continuously at his quest for the elucidation of red cell factors. It is fitting that his efforts should have been crowned with such success and that two of his former pupils, Philip Levine and Alexander S. Wiener, should have become pioneers and outstanding leaders in this field of investigation.

The great interest in this subject was well brought out in the Dallas Mexico City Congress of November 1946. Here were brought together many of the recognized leaders in this and related fields and the discussions which took place and which are reported in this special issue of Blood were highly stimulating. This was particularly true with regard to such controversial matters as that relating to nomenclature, the Fisher Race theory of three gene loci vs. the Wiener theory of multiple alleles, blocking antibodies vs. cryptagglutinoids, and other matters of present day importance. It cannot be said of the various individuals working in this field that they lack enthusiasm or desire to spread their various theses across the world's literature. Although some of their discussions have on occasion seemed to descend to acrimonious levels one cannot deny that they have often stimulated the interested investigators to renewed endeavors which have at times led to valuable discoveries.

What is occasionally lacking in immuno-hematologic discussions is an indication of their relationship to hematologic events in general and to the pathogenetic aspects of hemolytic disease in particular. Erythroblastosis fetalis is no more nor less than an acute hemolytic disease (of the newborn) which can be well understood in almost all its ramifications once one is acquainted with the general hemolytic mechanisms as induced by immune antibodies and other materials. The results obtained in the experimental production of various types of hemolytic anemia[1] using immune antibodies fitted in perfectly with the concept of iso-immunization with the Rh factor as later advanced by Levine and his co-workers.[2] In a recent article by the French worker Bessis[3] the close relationship of the experimental hemolytic anemias produced by anti-sera to the clinical syndrome of erythroblastosis fetalis is well brought out, clinical, hematologic and histopathologic features being described in detail.

The meetings on the Rh factor led to a sympathetic understanding of the different problems of investigators in their respective environments and thus to a definite feeling that international meetings were something to be carefully fostered. The Dallas Mexico City meetings were originally conceived as little get togethers

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to discuss the Rh factor Like many events which happen in Texas, it expanded —almost spontaneously it would seem—into a relatively large Congress and out of it has come the International Society of Hematology This group bids fair to be a potent force in the constantly expanding field of disorders of the blood and blood forming organs

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A SURVEY OF THE SIGNIFICANCE OF THE Rh FACTOR

By Philip Levine, M D

FOLLOWING Landsteiner s brilliant description in 1900–1901[1, 2] of the four blood groups, transfusions gradually became on the whole a safe and routine procedure However intragroup transfusion reactions were still observed in two groups of cases (1) patients who had previously received a series of uneventful transfusions and (2) women at the very first transfusion associated with pregnancy It was for a long time suspected that differences other than the 4 blood groups were responsible for isoimmunization and the production of new antibodies, but the actual demonstration of immune isoagglutinins was very seldom observed In the course of the study of this material an entirely new field of clinical importance was opened by the discovery of the role of fetal blood in isoimmunization, a subject hitherto of theoretical interest only

HISTORICAL DEVELOPMENTS

In 1900 two other discoveries pertinent to isoimmunization and erythroblastosis fetalis were made The first was the description by Ehrlich and Morgenroth[3] of the phenomenon of isoimmunization in goats and later extended by other workers to many species of animals In general the procedure used was cross transfusion within any particular species which resulted in the appearance of immune iso agglutinins or isohemolysins This work led to the concept of the individuality of animal blood by virtue of combination and permutation of a number of antigenic and hereditary substances in the red blood cells [4] In 1900 the Mendelian laws of heredity published 35 years previously, were independently rediscovered by Correns Tchermak and de Vries With the description of the human factors M N and P in 1928 the concept of the individuality of blood was extended to include human blood also although in these discoveries Landsteiner and Levine used heteroimmune sera [5]

In 1939 Levine and Stetson[6] offered an explanation for the origin of an atypical agglutinin held to be responsible for a severe transfusion reaction in a recently (1937) delivered woman at her very first transfusion The serum of this patient who had just delivered a macerated fetus agglutinated the cells of 80 per cent of group O individuals The intragroup agglutinin was at least as active at 37° C as at room temperature and in this respect it differed widely from the normal atypical iso agglutinins (anti A_1 anti O and anti P) studied extensively by Landsteiner and Levine [7]

Levine and Stetson assumed that the fetus inherited a dominant agglutinable factor from the father but not present in the mother s blood Isoimmunization could then result from the transplacental transfer of minute quantities of fetal red blood cells and/or tissue cells into the maternal circulation

The authors were led to suggest transplacental isoimmunization because of paral

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lel observations made by Irwin[8] Gorer,[9] and Lumsden[10] Irwin demonstrated mutual isoimmunization in back cross hybrids of two species of doves joined by parabiosis Gorer described the appearance of immune isoagglutinins in certain strains of mice following transplantation of a mouse sarcoma and an identical finding was reported by Lumsden in rats The latter 2 workers noted necrosis of the transplants in certain animals which was attributed to the development in the host of immune isoagglutinins directed against antigenic factors in the red cells of the donor transplants

It was this concept of placental isoimmunization which paved the way for the subsequent findings on the pathogenesis of erythroblastosis At the same time Levine and Stetson described a new blood factor which was independent of the other hitherto known blood properties such as A B M, N and P However, no name was assigned to this new blood factor which was antigenic in the same species (isoimmunization), but not antigenic or as we know at present poorly antigenic in animals (heteroimmunization)

At about the same time (1937) Landsteiner and Wiener[11] were investigating a factor in the red blood cell of the rhesus monkey related to but not identical with the human M factor In the course of their studies of the antibodies in sera produced in animals injected with rhesus blood another human blood factor was discovered which they called Rh [1 13] As in the case of the M factor it indicated a property in rhesus blood related to but not identical with the factor in human blood

At first these workers did not suspect that their heteroimmune agglutinin derived from animals was identical in specificity with the isoimmune antibody found in the patient studied by Levine and Stetson Because the serum was derived from the experimental animal Landsteiner and Wiener had no way of knowing that their factor was important clinically Later in 1940 however Wiener and Peters[14] observed that the Rh factor was antigenic in Rh individuals who had been transfused several times with Rh+ blood * These workers now suspected that the human Rh factor was probably identical with the unnamed factor previously described by Levine and Stetson

Later in 1940 Levine and Katzin[15] studied several cases similar to the one described with Stetson In each case there was a severe or fatal transfusion reaction at the very first transfusion in a woman who had recently delivered As in the remarkable case of Zacho[16] these agglutinins were more active at 37°C than at lower temperatures Accordingly Levine Burnham and Katzin[17] referred to them as warm agglutinins The obstetrical histories of this group of women were striking because of the high incidence of fetal and neonatal morbidity and it was suggested that the phenomenon of immunization with fetal blood responsible for intra group transfusion reactions was directly correlated with the fetal and neonatal morbidity When the histories revealed that these infants suffered from one or another form of erythroblastosis fetalis it was suggested that the intra uterine

It is of interest that all their anti Rh sera were of the cold variety and the authors suggested a compatibility test with incubation at icebox temperature In the light of our present knowledge it is probable that these sera contained blocking antibodies

blood destruction was brought about by the action of the maternal antibodies which found their way into the fetal circulation to react with and destroy the infants Rh+ blood [18, 19]

STATISTICAL PROOF

The proof of the concept presented is indicated in table 1 which shows the striking statistical differences in a series of mothers of erythroblastotic infants as compared with a random white population

Further proof was supplied in a demonstration that the incidence of the disease in any given population depends upon the incidence of Rh− individuals in the test with anti Rh_0 (anti D) agglutinin

TABLE 1 *—*Statistical Proof*

	Per cent	
	Rh+	Rh−
Random population male or female	85	15
350 mothers of erythroblastotic infants	10	90
204 husbands of Rh− mothers	100	—
139 affected infants of Rh− mothers	100	—

* After Levine[20]

TABLE 2*

Race	Number tested	+ (%)	− (%)	Incidence of Erythroblastosis fetalis
White[19]	334	85 0	15 0	2 1
Negro[22]	264	95 5	4 5	1 7
American Indian[23]	120	99 2	0 8	?
Chinese[24]	150	99 3	0 7	very rare
Japanese[25]	150	98 0	2 0	very rare

* After Levine[21]

In 1941–1943 Levine and his coworkers established the following additional facts

1 In the 8 per cent Rh+ mothers of erythroblastotic infants the isoimmunization was attributed to finer differences of the Rh factor (anti C) * to a new blood factor called Hr (genetically related to Rh) and to the factors A and B [19–6]

2 The Rh factor was presumably limited to red blood cells [7] Certainly in the affected infants the Rh factor could not be demonstrated in the body fluids

3 Anti Rh agglutinins could be demonstrated in less than 50 per cent of the Rh− mothers of erythroblastotic infants [19] It was assumed that in the remaining cases the hemolytic process was induced by the action of an antibody of another variety

* For a discussion of the terminology see page 7

not detectable by methods hitherto employed [8] (In 1944 and 1945, Race [29] Wiener [30] and Diamond [31] independently described the blocking antibody)

4 Intra group transfusion accidents could be prevented by the administration of Rh− blood to Rh− patients [19]

5 Anti Rh sera differ in specificity 2 of them now called anti D (anti Rh_0) and anti C (anti Rh′) giving 4 types of reactions [3] The anti Hr serum (anti c) and anti Rh′ (anti C) gave only 3 types of reactions since a blood failing to react with both sera was not found

6 The final statistical study revealed the far greater importance of the anti D serum presumably because the D (Rh_0) factor was more antigenic [19]

7 Human anti Rh sera were superior to the experimental serum produced in animals by injections of either rhesus [1, 13] or human blood [33, 34] Accordingly the experimental serum was largely abandoned at a very early date

8 The hemolytic process in the affected infant could be treated more effectively by transfusion of Rh− blood since the transfused Rh+ blood as well as the infant s own Rh+ blood were still subject to hemolysis in the neonatal period [19]

9 Several factors of safety were listed which were responsible for the com paratively low incidence of erythroblastosis fetalis in spite of a high frequency of incompatible mating ($85 \times 15 = 12\ 75$ per cent) (a) the current tendency to small families (b) a high incidence of heterozygous fathers and (c) the failure of many Rh− women to produce anti Rh antibodies It was suggested that the capacity to produce antibodies is dependent upon one or more genetic factors [19, 35]

In the 6 year interval since the description of the pathogenesis of erythroblas tosis fetalis some notable contributions were made by the British workers Fisher Race and their colleagues Taylor Coombs and Mourant, and by the American workers Wiener Diamond Witebsky Chown Hill and Haberman These dealt mainly with finer methods for detection of immunization theories on the genetics of Rh Hr system attempts to enlarge the supply of human anti Rh sera for diag nostic purposes and replacement transfusion of the affected infant

GENETICS OF THE Rh Hr SYSTEM

Reference has already been made to the 4 types of reactions given by two anti Rh sera (anti D and anti C) in striking contrast to 3 types of reactions observed on testing several hundred bloods with anti C and anti c Because of the historical significance of these facts and their bearing on the linkage theory of Fisher table 3 showing the relationship of these 3 sera is reproduced

Two of the 3 sera anti D and anti C were produced by Rh− mothers of eryth roblastotic infants while anti c (anti Hr′) was observed in an immunized Rh+ mother [19, 40] In the latter case the husband was Rh− but the mother s serum satis fied the criteria of isoimmunization i e a factor in the blood of the husband and affected infant not present in the mother s blood and causing the production of specific antibodies by the mother

Because anti C and anti c gave only 3 types of reactions it was suspected that the genetic relationship of factors C and c (Rh and Hr) was analogous to that ob served by Landsteiner and Levine for M and N i e 2 allelomorphic genes at a

particular locus on a chromosome [41] It is for this reason that the 2 letters "Rh" were reversed to yield the term "Hr" for the new blood factor Accordingly, the 3 serologic types correspond to the 3 genotypes (1) CC, homozygous, (2) Cc heterozygous and (3) cc homozygous The sum of the first 2 gives the incidence of positive reactions with anti C (about 70 per cent) and the sum of the second and third gives the number of positive reactions with anti c or anti Hr′ (about 80 per cent)

It is true that Levine s original anti Hr′ serum (anti c) was of weak activity but the incidence of the factor could have been calculated by taking into account the incidence of positive and negative reactions with anti C which was maximally active Levine however, was unable to explain the genetics of the factor D determined by reactions with anti Rh_0 (anti D) or the factor E discovered independently by Race[42] and Wiener [43]

TABLE 3 *—*The Cross Roads Experiment*

Based on Tests with 334 Random Bloods (White) Carried out in April May and June 1941†

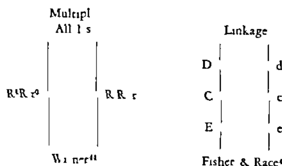
Terminology of Wiener and Landsteiner	Mrs M F anti Rh	Mrs M S anti Rh	Mrs K F anti Hr	Incidence of type (per cent)
Rh_1	+	+	o or ±	71
Rh_2	+	o	+	71
Rh′	o	+	o or ±	2
Rh−	o	o	+	13

* After Levine[39]

† At the request of Dr Wiener the scheme of the reactions indicated was made available to him for inclusion in the third edition of his book Blood Groups and Transfusion pp -53–254 (C C Thomas)

Without taking into account the Hr factor Wiener suggested that the 4 types of reactions could be explained on the basis of multiple alleles at first 3 genes and after the description of the E factor 6 genes *

Subsequently Fisher and Race[4] suggested the alternative theory of linkage at 3 different loci on a particular chromosome The two contrasting theories are illustrated below



Fisher produced some genetic evidence to indicate that the gene C is located in a position intermediate between D and E

* For a discussion of this theory see Wiener [44]

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some * It must be stated, however, that geneticists always find it difficult to differentiate multiple alleles from closely linked genes

Because genetic usage requires variations of a symbol for allelic genes at a given locus, the linkage theory necessitates a departure from the term Rh as a gene Accordingly, Fisher arbitrarily selected the letters C, D, E for the Rh genes and c, d e for the corresponding Hr genes There is no reason for discarding the terms Rh and Hr as blood factors

In a sense the choice of D for the factor described by anti Rh_0 serum is most fortunate The student in this field can readily orient himself since the anti D serum, already referred to in the literature as the diagnostic serum is clinically the most important one because of the greater antigenicity of the D factor In terms of the linkage theory an Rh+ individual is one whose blood contains the factor D as indicated by a positive reaction with anti D, the diagnostic serum (anti Rh_0) Such individuals may also possess the factor C i e , DCe or Rh_1 or the factor E i e either DcE or Rh or all 3, i e , DCE or Rh_1Rh_2 An Rh_0 individual is represented as Dce since his blood will react with only anti Rh_0 serum and with 2 anti Hr sera By the same token an Rh− individual is any one whose blood fails to react with anti D The several possibilities are given below

Rh−	Rh′	dCe
	Rh″	dcE
	Rh′ Rh″	dCE
	Rh negative	dce

In this paper the term Rh− indicates the composite group while Rh negative refers to the absence of all Rh factors and by the same token the presence of all 3 Hr factors

TABLE 4

anti		anti	
D		d	
	or		
C		c	
	or		
E		e	
+		+	heterozygous
+		o	homozygous
o		+	homozygous

These considerations are important for the clinician since they aid in the differentiation of Rh+ husbands as homozygous (DD) or heterozygous (Dd) Obviously the prognosis in pregnancies is far better for matings in which the husband is heterozygous since 50 per cent of the offspring will be Rh− (dd) and therefore incapable of immunizing the Rh− mother (Dd x dd) With 2 genes at a particular locus serologic tests for the corresponding blood factors reveal directly the 3 phenotypes (serologic types) corresponding to the 3 genotypes as shown in table 4

* For an excellent summary of the Fisher theory see Race [18]

Accordingly Fisher extended the concept of the MN type of relationship to include genes for the 2 remaining Rh factors D and E In doing so he had to postulate the existence of 2 additional Hr genes d and e allelomorphic respectively with genes D and E One of these e was subsequently discovered by Mourant[49] when he described a new human antibody which gave 96 per cent positive and 4 per cent negative reactions As will be shown below these figures correspond almost exactly to the theoretical values derived from a calculation of gene frequencies

The existence of the third variety of anti Hr antibodies anti-d, has not yet been definitely established This, however, does not destroy the validity of the theory since with rare exceptions all anti Hr sera can be produced by the much smaller group of 8 per cent Rh+ mothers of affected infants or Rh+ individuals immunized by transfusion of Hr+ blood In contrast to the 92 per cent Rh− mothers who produce anti Rh antibodies only those homozygous for D (DD) can produce anti d

Apparently the varying incidence of the several antibodies observed is an index of the degree of antigenicity of the corresponding factors since the number of incompatible matings cannot differ for factors determined by allelomorphic genes These considerations are illustrated below

	Immunized Mothers		*Antibodies Produced*
92%	Rh−	(cde)	anti D (also anti C and anti E)
	Rh−	(Cde)	anti D (also anti E and anti c)
	Rh−	(CdE)	anti D (also anti-c and anti-e)
8%	Rh+	DD	anti d
	Rh+	CC	anti c
	Rh+	EE	anti e

This scheme is simplified since the Rh+ mothers are represented as being homozygous for a particular Rh factor

The incidence of matings incompatible for the 3 Rh and Hr factors is given as follows

Rh of Mother (anti D)	Husband × Wife	Incidence of Type (%)	Incidence Mating (%)	Antibodies Produced	Incidence of Antibody
−	D × d	85 × 15	13	anti D	frequent
+	C × c	73 × 27	20	anti C	rare
+	E × e	30 × 70	21	anti E	occasional
+	d × D	63 × 37	23	anti d	?
+	c × C	80 × 20	16	anti c	rare
+	e × E	97 × 3	3	anti e	very rare

Wiener's view[44] expressed in numerous papers that the genetics of the Rh factor was based on a series of multiple alleles at 1 locus does not take into account the existence of the Hr factors Furthermore it is difficult to incorporate into the multiple allelic theory such new Rh genes as those described by Stratton[46] (D^u) and Race[47] (C^w) The complex antigenic structure of the Rh factor can be more readily explained in terms of linkage at several loci along the length of the chromo

an anti E agglutinin was found and curiously enough anti C serum was first used late in 1943 and published in 1944 [31] Fortunately, Levine had available large quantities of potent specimens of anti D anti C and a weaker anti c in his first studies in 1941

Until anti-d sera become available there is no choice but to use anti c for differentiation of homozygous and heterozygous Rh+ husbands of Rh− mothers This is possible because there is a high degree of correlation of genotypes DD and CC for the 71 per cent of Rh+ individuals of type DCe and DCE (Rh_1 and Rh_1Rh_2) As first pointed out by Levine[5] anti-c cannot be used for the 14 per cent of Rh+ individuals of types DcE (Rh) or Dce (Rh_0) For this group an anti-d serum is essential

Through the use of the hemolytic effect of anti Rh and anti Hr serums in the presence of whole blood and complement Hill and Haberman[63, 97] have been able to show a difference in the hemolysis of homozygous and heterozygous bloods In the presence of the specific antiserum (anti C D or c) homozygous cells showed approximately twice the hemolysis observed in heterozygous bloods

SOME PRACTICAL CONSIDERATIONS

With the aid of anti D the diagnosis of erythroblastosis fetalis is established in 92 per cent of all cases if the mother s blood is negative (Rh−) and if her serum contains anti Rh antibodies (agglutinins or blocking antibodies) In the smaller group of 8 per cent Rh+ mothers, the blood should be submitted to special workers in the field for testing with the other anti Rh and anti Hr sera

The mother who has already delivered an affected infant should not become pregnant until an interval of several years elaspse long enough for residual antibodies to disappear and for a rest period for the antibody producing cells In the event of another Rh+ fetus in the next pregnancy it is possible that the degree of isoimmunization will not be so intense Should periodic tests show an increasing antibody production many authorities believe that labor should be induced in order to shorten the period of intra uterine blood destruction

Since antibodies have been shown to persist for a number of years further pregnancies are to all intent and purposes excluded especially for the older Rh− woman whose husband is homozygous With another pregnancy already in progress when antibodies residual from the preceding pregnancy have not yet disappeared it will be very difficult—if not impossible—to differentiate newly formed from residual antibodies particularly if the husband is heterozygous Reference will be made later to the value of the anti human globulin test on the cord blood but this test can be applied only after delivery (see page 13)

Until our knowledge is extended the number of pregnancies of Rh− women already immunized by transfusions and/or previous pregnancies should be limited In practice this is equally applicable to Rh+ women immunized by other factors Even though some of these infants will recover with replacement transfusions there is always the increasing danger of later complications because of kernicterus

In the case of Rh− women in general the number of pregnancies to be recom-

The Johannsen formula previously used by Landsteiner and Levine[41] for the genetics of the MN factors is generally applicable for all genetic systems in which there are 2 allelic genes and 3 types corresponding to 3 genotypes This useful formula is based on the application of the binomial theorem to the gene frequencies The frequency of any one gene is equal to the square root of the p rcentage of non reactors who must be homozygous

The brilliance of Fisher s contribution is seen from an application of Johannsen s formula to Mourant s[49] recent discovery of the e factor Anti E gives 30 per cent positive reactions and 70 per cent negative reactions in both English and American populations The 30 per cent containing the E factor consist of a certain percentage of homozygous EE and the remainder are heterozygous or Ee Application of the formula gives the following

Frequency of gene e = $\sqrt{70}$ = 8 36
Frequency of gene E = 10 − 8 36 = 1 64
EE = $(1\ 64)^2$ = 2 7
97 3% { Ee = 2 × 1 64 × 8 36 = 27 3 } 30%
{ ee = (8 36) = 70

Remarkably enough these derived values are in almost perfect agreement with the data obtained by Mourant for anti e i e 96 per cent These findings constitute very significant evidence in support of Fisher s linkage theory

TABLE 5 —*Determination of Gene Frequencies for the Rh Hr Systems (C c D d and E e)*

X = Incidence one dominant gene
x = Incidence of the other dominant gene

X + x = 10	$X + 2X + x^2 = 100$		
0 + 10	0	0	100
1 + 9	1	19	81
2 + 8	4	32	64
3 + 7	9	42	49
4 + 6	16	48	36
5 + 5	25	50	25
6 + 4	36	48	16
7 + 3	49	42	9
8 + 2	64	32	4
9 + 1	81	18	1
10 + 0	100	0	0

In dealing with immune isoagglutinins of human origin which occur very rarely it is not surprising that a particular antibody perhaps the only one available at the time will not give maximally potent reactions Although this occurred with the first anti Hr serum (anti c) nevertheless its genetic relationship with the factor C was obvious since anti C gave maximally potent reactions Because of these considerations the writer hesitated to publish at length on the Hr factor until a more potent serum became available

Historically it was of interest that anti D and the rare anti c sera were available to the British workers (Race and Taylor) for their first studies [50] Somewhat later

A direct reaction given by blocking sera was discovered by Diamond and Abelson [55] when they recommended the slide test and concentrated cell suspensions Subsequently, it developed that serum or plasma was essential as a suspending medium for the test cells and that the test could be carried out in test tubes, thus making it possible to carry out quantitative studies [56] A notable contribution was made by Diamond and Denton[57] when they demonstrated that bovine albumin was suitable for suspending the test blood

Wiener s application of the term conglutination [11] appears unfortunate particularly since there is no evidence indicating that many proteins serum, albumin, globulin and fibrinogen in the form so-called X protein are essential for the direct reactions In this connection it may be cited that certain concentrations of acacia Le Page s glue No 7 (by virtue of its acacia content) polyvinyl alcohol pectin and numerous other nonprotein material may be used to elicit the reaction [58 59] However, these are not as satisfactory as bovine albumin because of their tendency to form rouleaux formation With the addition of minute amounts of saline the rouleaux disappear and the specific reaction still remains (Levine and Wigod[59]) *

Another notable contribution was made by Coombs Mourant and Race[60 61] when they showed that after prolonged washing with saline Rh+ cells coated with blocking antibodies are specifically agglutinated by precipitins for human serum Presumably a layer of antibody is fixed to the surface of the red blood cells and on addition of the anti human globulin serum the specific union with the coated red cells results in varying degrees of agglutination depending upon the intensity of the coating The British workers and Haberman and Hill[63 63] found this test to be most useful in determining whether or not the infant s cells at delivery had been sensitized with mother s blocking antibodies In a number of instances the author successfully applied this test to differentiate Rh+ cord blood from genetically Rh− blood exposed to but not damaged by maternal antibodies residual from the preceding pregnancy The degree of coating can be determined by quantitative studies to determine the greatest dilution of anti human globulin which will still agglutinate the washed blood Of the several procedures to detect coated Rh+ cells the anti human globulin test is by far the most sensitive and it should become a routine procedure for testing the red cells obtained from cord blood This reaction could safely be used as a guide for therapy and in the event of intense agglutination replacement transfusion should be carried out

Attempts were made by Wiener[11] to correlate the clinical picture in the affected infant with the presence of agglutinins or blocking antibodies Hemolytic symptoms were associated with blocking antibodies while agglutinins were held to be responsible for severe jaundice general toxicity and kernicterus The claim was made that kernicterus was the end result of thrombi (consisting of specifically

In titration of blocking antibodies the author uses pooled male s rum as a dilu nt and albumin suspended Rh+ cells [29] Under these conditions much higher titers are obtained than by Wiener s original method (plasma both as a diluent and suspending medium) [99] The results are at least as good as those obtained in the modified test in which Wiener employs for the first time albumin solution (1 part of 25% human albumin and 4 parts oxalated human plasma) [100]

mended will depend upon the ease with which antibodies are produced With routine testing for antibody production with each pregnancy, the much smaller group of Rh— women who are readily immunized can be selected As already mentioned the capacity for antibody production is determined by one or more genetic factors In any event the incidence of erythroblastosis fetalis can be lowered by prevention of isoimmunization in all of our Rh— female population who may be candidates for transfusion or intramuscular injection of blood[53] (see page 17)

In view of the high fetal morbidity in intensively immunized Rh— women whose husbands are homozygous termination of accidental pregnancies by therapeutic abortion seems justified [39] In the several cases in which this recommendation was made the Rh— patient had potent antibodies residual from the preceding pregnancies Except for those immunized by previous transfusions, these women already had one or more normal Rh+ children and had lost one or more affected infants For this group of cases either artificial insemination or adoption may be recommended

AGGLUTININS AND BLOCKING ANTIBODIES

In the initial study on the pathogenesis of erythroblastosis fetalis anti Rh agglutinins were observed in less than 50 per cent of the Rh— mothers * It was obvious that the remaining Rh— mothers were immunized because their infants had hemolytic symptoms and these mothers were equally subject to severe transfusion reactions The view was expressed that antibodies capable of reacting in vivo cannot be demonstrated because of limitations in the sensitivity of the technic employed [5]

It is of interest that time honored methods for testing were used i e Rh+ cells suspended in saline after previous washing to remove serum elements Curiously enough the logical step of testing the mother s serum under the conditions existing in vivo i e Rh+ cells suspended in plasma was not taken

In 1944 Race [29] Wiener[30] and Diamond[31] independently described incomplete blocking or inhibiting antibodies produced by Rh— mothers whose serum failed to agglutinate saline suspensions of Rh+ cells The indirect method was employed so that 3 reagents were required for their demonstration The mother s serum presumably coated the Rh+ cells suspended in saline and such treated cells now became resistant to the action of anti D agglutinins

In a number of sera titration with saline suspended cells revealed the presence of a prozone in the higher concentrations and increasingly strong reactions on further dilution In the light of present knowledge these sera contained a weak blocking antibody and a stronger agglutinin It was shown that under certain conditions by absorption of undiluted sera the blocking antibody could be specifically removed and the anti Rh agglutinin could be recovered almost quantitatively [30, 54]

The indirect test however was tedious and time consuming because 2 incubation periods were required so that it is no longer employed as a routine procedure

Undoubtedly this figure is probably too high since in several cases weak reactions were elicited on the addition of an excess of serum Under these conditions the effect could be attributed to the action of the blocking antibody

duction by the immunized mother represent an ever changing configuration of the immune globulin which still retains its characteristic specificity

On the basis of discrepancies in the behavior of blocking antibody and the anti human globulin test for coated cells, Hill and Haberman[63] assumed the existence of a third order of antibodies

These authors classified the antibodies on the basis of saline agglutinins, blocking antibodies (those that saturate the Rh antigen without causing agglutination), and the cryptagglutinoids (those antibodies that do not agglutinate in saline, do not block but will be demonstrable by the anti human globulin test or in albumin and serum) They applied the term developing test to the use of the anti human globulin serum on fetal erythrocytes

In conclusion serologic tests are now available for a somewhat more accurate correlation of symptoms in the infant and antibody content of the mother s serum and infant s serum and red blood cells In the final analysis the intensity of the disease process and the therapy will be determined by serologic study of the cord blood particularly on the red blood cells However more intensive studies on the serological and physico chemical properties of purified preparations of the several varieties of antibodies are required for a fuller understanding of the subject

SPECIFIC THERAPY OF THE AFFECTED INFANT

As first suggested by Levine Burnham, Katzin and Vogel[19] the affected infant of an Rh− mother should be vigorously transfused with Rh− blood which is not subject to the action of stored maternal antibodies It is essential to maintain a hemoglobin level above 65–70 per cent In some cases it is necessary to transfuse repeatedly until the infant is temporarily Rh− by virtue of the normally surviving donors Rh− blood

In any event the infant s red cell after exposure to maternal antibodies is an injured one and is not apt to survive long in the infant s circulation One cannot underestimate the degree of blood destruction resulting from the action of passively transferred maternal antibodies stored presumably in the infant s tissue spaces In one severely affected infant reported elsewhere who was transfused several times maternal blocking antibodies were still demonstrable on the twenty fifth day of life At this time the infant s blood was Rh− by virtue of the surviving transfused cells On the thirty fourth day of life the infant s blood for the first time was agglutinated by anti D serum but it was still a mixture of about one third Rh+ and two thirds Rh−

More recently several workers (Wallerstein [64] Wiener [65] Diamond[66]) have been carrying out replacement transfusions by washing out the infant s circulation with large quantities of Rh− blood Wallerstein [64] Wiener[6] and Vogel[67] have been withdrawing the infant s blood either from the fontanelle or the radial artery and administering the donor s blood into one of the superficial veins * Units of 20 cc of infant s blood are withdrawn and replaced with 20 cc Rh− blood and the proc

* The method of choice seems to be the use of cord veins which can be cannulized with the aid of a special plastic catheter as suggested by Diamond [66]

agglutinated cells) in the arterioles of the liver causing severe icterus which supplied the characteristic coloring to certain areas of brain tissue whose arterioles were likewise plugged with agglutination thrombi Although these thrombi were found in numerous organs harmful effects were assumed to be specifically localized in the liver and brain The consensus is that the agglutination thrombi observed by Wiener and Brody represent not the specific lesion but rather postmortem changes

Many exceptions to these claims have occurred, i e the severe anemia associated with strong anti Rh agglutinins and no symptoms suggestive of kernicterus The latter condition has now been observed in a number of cases in which blocking antibodies alone were found

It is of course established that maternal blocking antibodies in contrast to anti Rh agglutinins pass into the fetal circulation and specifically unite with fetal blood Remarkably enough these cells remain unagglutinated in the fetal or infant s circulation although they are in continuous contact with antibodies in a medium of plasma and in a number of instances the antibody concentration in the infant s circulation is sufficiently great to indicate a state of equilibrium on both sides of the placental barrier In any event there is no justification for the use of the terms univalent and bivalent for blocking and agglutinating antibodies respectively Since both sorts of antibodies exert their harmful effects in vivo the difference seen in vitro would seem to become less significant The fact is that in vivo both sorts of antibodies are associated with the identical clinical entity of intense blood destruction The fact that blocking antibodies are frequently demonstrated in the infant s serum does not necessarily indicate that they are of smaller molecular size than agglutinins

It was further claimed by Wiener that agglutinins exert their harmful effect mainly at delivery and not during the latter part of the pregnancy It is difficult to accept the view that a concentration of maternal agglutinins sufficient to induce severe symptoms of blood destruction or icterus gravis could be attained from the process of parturition and delivery Certainly it can not be expected in the case of infants delivered by Cesarean section Furthermore the clinical picture is almost identical in many anemic infants of mothers with either agglutinins or blocking antibodies

In a number of instances qualitative tests for blocking antibodies with albumin suspended cells will be entirely negative but titration in normal human serum as a diluent will reveal a prozone i e gradually increasing reactions on further dilution This obviously is a serious source of error which can readily be detected with the aid of the anti human globulin test As in the ealier reports on prozone due to a mixture of agglutinins and blocking antibodies these findings indicate the presence of two varieties of blocking antibodies More recently this view was confirmed by the results of specific absorption experiments by Levine and Wigod [62] On treatment with Rh + but not with Rh − blood the prozone is specifically removed and titration of the absorbed serum now reveals gradually decreasing reactions Accordingly one may assume that the antibodies in the course of their pro-

transfusion with Rh+ blood Undoubtedly the replacement is never complete and because of the residual antibodies in the tissue spaces, the Rh+ blood will not survive as long as Rh− blood

It is advisable to take the erythroblastotic infant off breast milk because anti-Rh antibodies are frequently present in the mother's milk (Witebsky[91]) This precaution should certainly be taken for the more acutely ill infants even though it is not definitely established that maternal antibodies are absorbed from the intestinal mucosa, at least in appreciable amounts

In the series of affected infants delivered by immunized Rh+ mothers the infant should be transfused with group compatible Rh+ blood of the same Rh Hr subtype as the mother If the isoimmunization is induced by factors A or B (e g , mother O, infant A or B) the infant should be transfused with group O blood along with the soluble group A and B substances of Witebsky [95]

PREVENTION OF ISOIMMUNIZATION OF Rh− INDIVIDUALS

All Rh− individuals requiring blood transfusions should receive Rh− blood only In this way isoimmunization will be prevented and if present the clinician will be spared the trouble of treating the patient for transfusion anuria perhaps unsuccessfully

As pointed out by Levine [35, 53] once a patient is immunized the individual remains potentially immunized for the remainder of his or her natural life time This is most important in the case of young girls even as infants If Rh− girls are transfused indiscriminately as they have been in the past their chances many years later for having 1 or 2 normal Rh+ children are considerably diminished These women are thus deprived of the several factors of safety which tend to reduce the incidence of erythroblastosis fetalis so that even their first Rh+ infant may be lost as a macerated fetus stillbirth or the infant may have the more severe forms of erythroblastosis fetalis [53]

There is reason to believe that those women who were not transfused may in several instances have received intramuscular injections of blood a routine procedure in the days preceding the use of vitamin K or for prophylaxis against measles In a larger series of cases of erythroblastosis fetalis in the first born, soon to be published by Levine and Rosenfield [69] histories of intramuscular injection were elicited in several instances

Regardless of the influence of previous transfusions the occurrence of erythroblastosis fetalis in the first born has some bearing on the mechanism of transplacental isoimmunization Wiener [70] had assumed that fetal blood entered the maternal circulation only during labor and delivery Although there may be another antigenic stimulus at delivery the transfer of minute quantities of fetal blood in the latter half or third of pregnancy must be the determining factor even in those Rh− women who may have been transfused many years previously With intervals of 9-14 years between the antigenic stimulus of a transfusion and the first pregnancy one may well assume that the damage to the fetus or infant is not caused by residual antibodies which are evanescent in character but rather by renewed immunization On renewed contact with this antigen many years later the

ess is continued until the infant s circulation is washed out with 1 liter of Rh− blood An additional quantity of Rh− blood corresponding to 10 cc per pound should then be administered preferably at the beginning of the transfusion

In less severely affected infants as indicated by the serological tests on the cord red blood cells smaller volumes of Rh− blood may be used Past experience reveals that many affected infants recover after 1 or 2 single transfusions The radical replacement should be reserved for those infants who have been exposed to prolonged intrauterine blood destruction

From the point of view of prognosis the most significant information will be derived from a study of the cord blood carried out soon after delivery The presence of maternal antibodies in the cord serum is not as significant as the presence of maternal antibodies fixed to the infant s red blood cells In several cases the increase of antibody content in the mother proved to be misleading since the infant was shown to be Rh− The mechanism of such nonspecific antibody production is still to be investigated As indicated above genetically Rh− blood can be differentiated from specifically coated Rh+ cells with the aid of the reaction of Coombs Mourant and Race

Assuming the infant to be Rh+ the ratio of blocking antibody in the maternal and cord blood is of some prognostic significance With more intensive isoimmunization of long duration a state of equilibrium can be established on both sides of the placental barrier in which case the prognosis is not favorable By earlier induction of labor a more favorable ratio may be obtained There is reason to believe that the same conditions which favor passage of fetal elements into the maternal circulation, i e gradual thinning of the placental barrier in the last third of pregnancy are at the same time more favorable for passive transfer of maternal antibodies into the fetal tissues

The author has recently studied a number of cases in which the infant delivered by early induction of labor and given an immediate replacement transfusion recovered completely from the anemia although the infant in the preceding pregnancy had died of erythroblastosis fetalis The prognosis however so far as freedom from symptoms due to kernicterus is concerned must always be guarded since the damage to the brain may not become manifest for many months or even several years In at least one such instance the infant delivered 4 weeks early did not require more than one transfusion at birth and another in the fourth day of life Although completely recovered from the anemia this infant is now developing symptoms indicative of kernicterus However the damage to the brain whatever the cause may be is determined by intrauterine action rather than by stored antibodies acting during the neonatal period

The recent criticism by Darrow[68] and others of the use of Rh− blood does not seem valid at least not for the severely affected infant It is highly probable that the mildly affected infant will recover either without benefit of transfusions or despite the transfusion of Rh+ blood One is not justified in assuming that the burden placed on the mechanism for the disposal of large quantities of destroyed blood does not exert a deleterious effect on an anemic and jaundiced infant To a lesser degree perhaps the same objections are applicable also for the replacement

receive either Rh+ or Rh− blood Although certain Rh+ patients may be immunized by the subtypes of the Rh factors (C E) or by the Hr factors (d, c, e) this occurs very rarely In any event, it is not feasible to take into account all possible antigenic differences at least for transfusion requirements * However, Rh+ individuals should not receive Rh− blood for the very practical consideration that Rh− blood should be reserved for Rh− patients

A PUBLIC HEALTH PROGRAM

In 1941 immediately after the role of the Rh factor in the pathogenesis of erythroblastosis fetalis was established the writer drafted 2 rules which were discussed by the Board of Medical Control of the Blood Transfusion Association and public health authorities These follow

1 In all individuals receiving repeated transfusions in whom untoward (intra group) transfusion reactions have been noted tests for the Rh factor shall be performed before any subsequent transfusion No subsequent transfusion shall be given to any such recipient found to be Rh negative except from an Rh negative donor whose red blood cells are shown to be compatible with the recipient s serum at 37°C

2 No woman with an obstetrical history characterized by habitual abortion stillbirth macerated fetus or erythroblastosis fetalis shall receive a transfusion unless tests for the Rh factor have been made and then if her blood shall prove to be Rh negative such transfusions shall be made only from an Rh negative donor whose red blood cells shall have been shown to be compatible with the recipient s serum at 37 C

Although these preventive measures were generally approved, no official action could be taken because no assurance could be given at the time that sufficient quantities of potent anti Rh serum would be available

In the light of our present knowledge regarding the influence of indiscriminate intravenous or intramuscular injection of blood it now becomes necessary to modify the second proposal so that it may perhaps read as follows

No transfusions in a female of any age from infancy on may be carried out unless she has been tested for the Rh factor If found to be Rh negative in tests with potent standard diagnostic anti Rh_0 (or anti D) serums she must receive only Rh negative blood whether it be given intravenously subcutaneously or by intramuscular injection

The general adoption of these rules will result in a reduction of the incidence of erythroblastosis fetalis and a striking reduction of serious and fatal intra group transfusion reactions

The writer has been encouraging public health authorities to adopt a comprehensive program of Rh testing and in several states the measure is now under consideration For example there is the successful experience reported by Lee Van Saun and Brown [74] in Passaic County New Jersey Since many states already have compulsory premarital and prenatal tests, no statutory authority is required except perhaps to obtain financial support for the program

All workers in this field are agreed that the Rh test should be done routinely

* It remains to be seen whether or not identity of antigenic factors in red blood cells of donor and host for skin or tissue transplants will give more satisfactory results Theoretically at least given a sufficiently large number of antigenic differences the success of the transplant will depend upon proper selection of the donor

antibody producing cells of the reticulo-endothelial system respond more rapidly (anamnestic reaction)

It is obvious that the biologic test as recommended by Wiener,[71] i e the administration of small quantities of Rh+ blood will also serve to immunize More sensitive tests are now available for detection of immune antibodies specific for differences within the Rh− and Rh+ group or new blood factors other than Rh Incompatibility can be excluded if the patient s serum does not agglutinate the donor s cells suspended in his own serum plasma or bovine albumin

A woman who has delivered an erythroblastotic infant if transfused many years later may tolerate one transfusion of Rh+ blood * In any event this transfusion will restimulate antibody production so that the following transfusion may result in a severe reaction perhap with anuria This is another example of the so called anamnestic reaction

TABLE 6 —*Erythroblastosis Fetalis in th First Rh+ Infant in Rh− Women*

	Transfusion History	
	Positive	Negative
No cases	19	9
Severity of disease		
mild	1	5
severe	6	4
fetal death	12	0

Since erythroblastosis fetalis is the result of prolonged intrauterine blood destruction the number of immunized Rh− women exceeds the number of affected infants In other words an Rh− woman may have anti Rh antibodies and yet her Rh+ infant may be entirely normal because the antibody production may have started too late in the course of the pregnancy to allow for much if any intrauterine blood destruction However the next Rh+ infant will certainly be affected probably with an unexpectedly severe form of the disease In any event these Rh− women are always subject to severe transfusion reactions should they require blood even many years after their last pregnancy

Intra group transfusion reactions in Rh− male patients can be readily prevented because as a rule after a series of uneventful transfusions the patient eventually will have a slight chill or mild jaundice following a particular transfusion This should serve as a warning to carry out Rh tests and if found to be Rh− only Rh− blood should be used for all future transfusions Unless these precautionary measures are taken the following transfusion will result in a severe if not fatal hemolytic reaction [7, 73] On the whole the transfusion risks are far greater in women than in men

For transfusion purposes it is preferable to think in terms of 8 different types rather than the 4 blood groups with the provision that Rh+ individuals may

In one instance there was a delayed reaction probably attributable to traces of residual antibodies produced by a transfusion 4 years previously [28]

reduced so that there is no danger of untoward reactions from subsequent injections Undoubtedly all these procedures will also be carried out for the production of other varieties of antibodies (anti C anti E and their corresponding Hr antibodies)

Two additional sources of potent anti Rh sera may be derived from the recent program of concentrating weak anti Rh sera,[79] and by certain treatment of so-called prozone sera The latter sera contain both blocking antibodies and agglutinins and were therefore considered not suitable for diagnosis However, the blocking antibodies in such sera may be specifically absorbed without affecting to any considerable degree the activity of the recovered agglutinin (Levine and Waller[51])

Rarely, some of the measures mentioned above may be applicable also to patients immunized by repeated transfusions

In this connection it is appropriate to mention that anti Rh sera may be used more economically by employing the capillary tube method of Chown instead of test tubes For this procedure small quantities of sera may be used and the tests carried out by Dr Chown in my laboratory show that his method is almost as sensitive as the test tube method [80]

Recently the National Institute of Health has ruled that no anti Rh serum be released for distribution unless it has a titration value of at least 1 32 In other words a serum which has a titre of 1 320 may be diluted 10 times, but the diluent must be such as not to diminish the protein content below 25 per cent of the normal content Accordingly normal human serum of a group AB or bovine albumin may be used as the diluent (6 per cent for agglutinins and 30 per cent for blocking antibodies) *

The reagent of choice is the agglutinin which is active on blood cells suspended in saline

More recently blocking antibodies have been recommended, (slide test[55]) but the red cells to be tested must be suspended either in their own plasma serum or bovine albumin [54–57] For large scale work it is preferable to use test tubes rather than the slide test The main advantage in the use of blocking antibodies is their greater availability since most Rh− mothers of erythroblastotic infants produce blocking antibodies

MECHANISM OF TRANSPLACENTAL ISOIMMUNIZATION

In 1939 Levine and Stetson suggested that products of the fetus (red blood cells or tissue cells) containing a dominant hereditary property not present in the mother s blood could find their way into the maternal circulation and thus stimulate the mother to produce specific antibodies When the pathogenesis of erythroblastosis fetalis was described experiments were carried out to determine whether or not the Rh factor was present in a water soluble form Using saliva as an index it was established that the Rh factor unlike the secretor types of groups A and B was limited to the red blood cells Accordingly the question arose as to the mechanism which permitted formed elements the size of a red blood cell to penetrate

* The Minimum Requirements for Blood Grouping Serum and Anti Rh Typing Serum were released on December 16 1946 from the National Institute of Health Washington D C

on the prenatal rather than the premarital specimen Unfortunately the lay public has been unduly alarmed regarding Rh incompatibilities There is almost never any indication for a couple to break their engagement solely because the young woman is Rh− The only exception applies to those Rh− women who had already been immunized by transfusions of Rh+ blood

In addition to routine screening tests for the selection of Rh+ and Rh− mothers tests for the presence of active isoimmunization should be carried out While these tests should also be performed in a state wide program all hospital laboratories especially those with large obstetrical services should be prepared to carry out these comparatively simple procedures Unfortunately there are altogether too few workers with sufficient experience or background in this new and clinically im portant field Certainly each of the larger hospitals should have at least one or more of their workers specially trained in all aspects of this field It is further sug gested that smaller hospitals pool their interests in organizing a properly equipped laboratory to serve the interests of their local community Such a program can conveniently be organized in conjunction with a central blood bank [75]

In recommending a public health program it is pertinent to mention that the morbid effects incidental to isoimmunization i e erythroblastosis fetalis and intra group transfusion accidents are observed far more frequently than those resulting from syphilis Obviously the element of contagion is not present in the case of isoimmunization The potential danger of Rh incompatibility varies in different races and is directly proportional to the incidence of Rh− individuals in any given population

A SUPPLY OF POTENT ANTI Rh SERA

If Rh tests are to be done on a broad basis in all pregnant women and all patients prior to transfusion requirements it is important to maintain a continuous supply of potent human anti Rh sera Unfortunately the experimental serum produced in animals injected with rhesus or human blood is not sufficiently potent and its use was largely abandoned soon after the description of the phenomenon of trans placental isoimmunization Even if an animal serum is produced in the future it will still be necessary to collect and store large quantities of human sera containing the other Rh and Hr antibodies and any other of the very rare sera containing antibodies of unusual specificities

In the past the outlook for large quantities of human anti Rh sera was not favorable More recently however a number of steps have been taken which should solve the problem of supply With routine testing a greater number of im munized Rh− women will become available Physicians should encourage these women to submit to periodic bleedings followed by replacement transfusion of Rh− blood In selected women who do not plan further pregnancies and in isoim munized men the potency of the antibody can be maintained by the intravenous injection of minute quantities of Rh+ blood insufficient to induce unpleasant reactions (Hill and Haberman[76]) Both Wiener[77] and Diamond [78] and others re port the immunization of Rh− donors on a voluntary basis by repeated adminis tration of Rh+ blood When antibodies begin to appear the dosage is appreciably

fully developed, fetus More recent data do not support the view that isoimmunization by the Rh factor per se plays any role in early fetal death If there is a higher than normal incidence of miscarriages in mothers of erythroblastotic infants this may possibly result from the effects of isoimmunization from the preceding pregnancies At any rate this subject merits further investigation

Erythrocytes can be observed in the yolk sac in the 4 weeks old fetus, and agglutinable properties have been demonstrated in the blood of the fetus, between the second and third months [85] There is reason to suspect that the more fundamental property of antigenicity and the capacity to unite with antibodies may be inherent even in the forerunners of the red cells Nevertheless isoimmunization by the Rh factor probably is not initiated until the latter half of the pregnancy when the blood vessels in the villi gradually approach the maternal sinuses and are in intimate contact over an ever increasing surface area Incidentally, pregnancy offers certain conditions which are peculiarly favorable to isoimmunization, i e slow administration of the antigen over a long period [86]

This concept of the mechanism of isoimmunization is compatible with the clinical observation that once an Rh− mother is immunized the condition is likely to recur in all succeeding pregnancies in which the fetus is Rh+ Apparently the isoimmunization is renewed even if an interval of several years elapses between pregnancies Furthermore the erythroblastosis is likely to be increasingly severe in successive pregnancies

It has been observed on clinical evidence alone that erythroblastosis fetalis occurs about once in every 438 deliveries If Rh tests are done in all cases of fetal and neonatal morbidity one can assume an incidence of about 1 150 to 1 200 deliveries Even this value is out of proportion to the number of pregnancies in the 13 per cent of susceptible matings i e Rh+ father × Rh− mother Accordingly it is necessary to assume several factors of safety (1) the current tendency to small families, (2) inability of many Rh− women to produce antibodies and (3) the high incidence of heterozygous fathers Reference already has been made to a recommendation which should decrease the incidence of erythroblastosis fetalis in the first born particularly in its more fatal forms

At present there is no specific measure which will prevent either the transfer of fetal blood across the placenta or the formation of antibodies on the part of the mother Possibly the injection of serologically active haptenes extracted from Rh+ blood may neutralize the action of antibodies as they are formed Such haptenes lack the property of stimulating antibody formation, but are capable of specifically neutralizing antibodies However the extraction of such haptenes from Rh+ material presents many technical difficulties [87]

The suggestion has been made recently that the injection of typhoid vaccine in the course of pregnancy may prevent or delay antibody formation on the part of the Rh− mother [88] However the experimental animal when injected with a mixture of numerous antigens responds with the production of a corresponding multiplicity of antibodies It is also conceivable that injection of nonspecific material may serve to stimulate rather than to depress antibody formation If this suggestion is to be given a fair trial it is preferable to inject the Rh− women with

the placental barrier in sufficient quantity to stimulate antibody production on the part of the mother

If the Rh factor were present also in a water soluble form the material could readily enter the maternal circulation in sufficient quantity to immunize In that event the maternal antibodies after passage into the fetal circulation would be completely inactivated by the excess of Rh soluble material thus preventing the antibody from uniting with the Rh factor in the red blood cells Accordingly the hemolytic nature of erythroblastosis fetalis substantiates the finding of Levine and Katzin that the Rh factor is present in the red blood cells, presumably in a water insoluble form Possibly the Rh factor may also be present in tissue cells, but this observation of Boorman and Dodd[81] is still to be confirmed

Witebsky[87] showed that Rh soluble material may be present in very small quantities in the amniotic fluid of some but not all Rh+ fetuses However this observation can have no bearing on the pathogenesis of erythroblastosis since each of the affected infants belonged to the so-called non secretor type

It does not seem necessary to assume the presence of gross lesions in the placenta which would have to recur and become operative in each succeeding pregnancy with an Rh+ fetus but not with an Rh− fetus It is significant that in the vast majority of the cases the course of the pregnancy and the delivery of these mothers is entirely normal Although there is no direct proof that the fetal red blood cells (a large formed element) find their way into the maternal circulation nevertheless the statistical data on the pathogenesis of erythroblastosis fetalis permit of no other conclusion

If one assumes that minute quantities of fetal blood either as intact red blood cells or as stroma pass the placental barrier this must occur in every normal pregnancy Isoimmunization may occur only if the fetus is Rh+ and the Rh− mother is genetically capable of producing antibodies Accordingly it becomes superfluous to assume the existence of genes determining placental permeability to formed elements *

It is well known to the immunologist that remarkably minute amounts of antigenic material (soluble proteins suspensions of bacteria or red blood cells) suffice to induce immunization In recent experiments in rabbits[88] distinct increases in agglutinin titre were observed following 14 daily injections of 2 cc of a 1 5000 suspension of human blood the total volume of which was 0 0056 cc whole blood The corresponding value for a woman weighing 120 pounds is only 0 13 cc

It will be recalled that in the latter part of the pregnancy when isoimmunization is believed to begin the blood vessels in the fetal villi are adjacent to the maternal sinus and separated from it by a single layer of cells It has been calculated by Dodds[83] and Dees Mattingly[84] that the total area of fetal villi of the human term placenta exposed to maternal sinuses is 70–120 sq ft and total length of these villi if laid end to end would measure 11 4 miles One fourth or more of the fetal blood is outside the fetus and in the placenta

In this connection it is significant that the pathological effects of isoimmunization by the Rh factor are observed exclusively in the fully developed or almost

* Cf Haldane[86]

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such antigens as tetanus and diphtheria toxoid and pertussis vaccine so that the mothers are at the same time producing antibodies which will be beneficial for their infants

Erythroblastosis fetalis assumes importance altogether out of proportion to its low incidence because it is the first example in any species of a new cause of fetal and neonatal morbidity i e , genetic differences involving a particular blood fa tor which has a normal incidence in any racial group Undoubtedly many examples *will be found in veterinary medicine at least in those species which are charac* terized by a placenta which does not differ radically from that in man

The essential feature of this form of fetal or neonatal morbidity is its selective effect on Rh+ offspring Thus in the case of twins only one of whom is affected the normal member is always Rh− Undoubtedly the mechanism of isoimmuniza tion may be operative in cases of selective fetal death in many animal species As examples may be cited the observations of Corner[89] and Robinson[90] on the cause of selective intrauterine death in pigs and ferrets These workers described multiple births in uteri which are normal in all respects and yet they harbor dead and nor mal fetuses lying side by side On the basis of the findings in man one is tempted *to speculate that the dead fetuses have a particular blood property derived from* the male parent which is absent from the blood of the normal fetuses and their mother

It is significant that the method employed to supply the evidence for the pathogenesis of erythroblastosis fetalis is mainly statistical Accordingly the same procedure may be used to determine whether or not isoimmunization by fetal blood (Rh or any blood factor) may or may not play a role in complications of pregnancy or other conditions of the fetal and neonatal period [91] Some preliminary findings on the role of A and B factors in causing stillbirths other than that due to erythro blastosis fetalis have already been published [86] In this connection mention may also be made of the recent findings of Yannet[9] and Snyder[93] on the high incidence of Rh negative mothers of infants and children affected with the so-called mental insufficiencies of the undifferentiated group The possible relationship of these cases to the after effects of kernicterus is still to be determined

ADDENDUM

In this contribution the author made reference to several publications which appeared after this paper was delivered at Dallas (November 1946)

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THE Rh GENOTYPES AND FISHER'S THEORY

By R R RACE, M R C S , L R C P

THERE are now growing up very many young people who owe their lives to the researches of Dr Levine,[1] the late Dr Landsteiner and Dr Wiener [2] I would like to express the thanks of another group of people, the English blood transfusion workers whose lives have not been saved, but have been made more interesting and exciting by the discovery of the Rh blood groups and their clinical associations In England the news of the discovery came like sunlight in the drab endless routine of wartime transfusion work

I am going to try and give you an account of our researches into the subgroups of Rh

As early as 1941 Landsteiner and Wiener reported an abnormal anti Rh serum which agglutinated the blood of only 70 of the 85 per cent of Rh positive people and it was supposed that there must be subtypes within the 85 per cent of Rh positives like those found in the ABO system Levine, also in 1941 found another abnormal anti Rh serum which was called anti Hr because it agglutinated all Rh negative bloods and which also agglutinated some Rh positive bloods These two observations made it certain that the Rh blood groups were much more complicated than had at first appeared

In 1943 owing to the very generous attitude of my chief the late Dr G L Taylor I was released from routine responsibilities and able to investigate an abnormal anti Rh serum which we had received from Dr McCall of Stoke on Trent Lancashire [1] The serum was from the Rh positive mother of erythroblastotic children Like Levine s anti Hr it agglutinated Rh negative blood but it agglutinated more Rh positives than did the anti Hr serum This serum which was called temporarily St agglutinated 80 per cent of English bloods [2] As it agglutinated all Rh negatives that is rr bloods it seemed reasonable to expect that it would also agglutinate bloods representing a single dose of the r gene that is to say heterozygous Rr bloods This was found to be the case Heterozygous Rr bloods could be disclosed by family Rh studies but not at that time recognized serologically Any Rh positive person who had an Rh negative parent or child must be heterozygous Rr Many such people were found and their blood was invariably agglutinated by the St serum The frequency of Rh negative bloods was about 15 per cent and the frequency of heterozygous Rr bloods could be calculated as about 48 per cent But 15 per cent + 48 per cent equals only 63 per cent and the St serum agglutinated 80 per cent Therefore some of the RR homozygotes were being agglutinated by St and some were not

This was of clinical importance for if blood were St negative it must be homozygous RR, and a father of erythroblastotic children if he were St negative, could not hope for any Rh negative children in the future More than half of Rh

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According to the theory, blood reacting $++-$ was R_1r, and this was verified in 2 ways R_1R_1 people could be recognized $+-$, and so could rr, $-+$ Blood was collected from the offspring of numerous matings of the type $R_1R_1 \times rr$, all such children must be R_1r, and their blood constantly gave the reaction $++-$ Secondly the expected frequency of R_1r in the general population, calculated from the $+-$ and $-+$ group was always extremely close to the observed frequency of $++-$ bloods

So now there were 6 serologically recognizable groups, $+--$ $+-+$, $+++$ etc and 5 genes The names I am using for the genes are not all the original ones, some were later altered to fit in with Wiener s scheme The other genotypes of the rare R″ and R_z genes are not shown as this complicates the table rather too much

TABLE 2 —*Combined use of three anti Rh sera*

	Original 85%	St 80%	K J 30%
	RR	− R_1R_1	− R_1R_1
			+ R_1R rare
	+ Rr	+ R_1R_2, R_2R_2, R_2r, R_1r	+ R_1R_2 & R_2R_2, R_2r
			− R_1r
	− rr	+ rr	− rr
			+ R r rare
Serological groups	2	3	6
Genes	2	3	5

genes	Anti Rh 85	80	30
R_1	+	−	−
R	?	−	+
R_2	+	+	+
r	−	+	−
R″	−	+	+

The reactions of the 5 genes could be tabulated These reactions are purely theoretical they show how red cells would react if their antigenic makeup depended on 1 gene instead of 2 In actuality, of course blood cells like other body cells, are controlled by a double set of chromosomes which includes 2 of the Rh chromosomes and a specimen of blood gives the superimposed reactions of these 2 Rh chromosomes As R_1R_1 reacted $+--$ these presumably would be the reactions of R_1 if it could be observed acting alone (in a haploid human, if such existed) Similarly rr reacted $-+-$ and presumably r alone would react $-+-$ The determination of the reactions of some of the other genes was sometimes a little more complicated For example blood of the genotype R′ r reacts $-++$ and the gene r reacts $-+-$ therefore the gene R″ must be negative with the 85 per cent serum and positive with the 30 per cent serum but without further information one could not say what the reaction with the 80 per cent serum would be The + reaction given by the R″r

positive homozygotes could therefore be recognized as such by the combined use of the original 85 per cent serum and the St serum

There were then 2 kinds of RR blood, one St negative and the other St positive and presumably there were 2 forms of the R gene responsible for manufacturing 2 kinds of Rh+ antigen The St negative form of the gene we called R_1 and the St positive form R , and these I supposed were the genotypes embraced by the 3 reaction groups or phenotypes +− containing only R_1R_1 ++ containing R_1R R_2R_2 R_1r and R_2r and −+ containing rr

The reactions of the 3 genes could be tabulated as shown in table 1 I will speak a little later about this jump from the reaction of actual blood representing the combined reactions of 2 genes to the hypothetical reaction that blood representing 1 gene would give

TABLE 1 —*Combined use of two anti Rh sera*

O d nary Anti Rh 85%		St Serum 80%		Inte pretation
		−	20%	R_1R_1
	37% RR			
		+	17%	R_1R & R_2R_2
+ 85% calculated				
	48% Rr +	} 15% + 48% = 63%		R_1r & R_2r
− 15%	15% rr +			rr

		Anti Rh sera 85%	80%
Genes	R_1	+	−
	R_2	+	+
	r	−	+

Most fortunately just at this stage in the work 2 identical anti Rh sera were sent one from Miss Boorman and Miss Dodd of the S W London Blood Supply Depot and the other from Dr C V Harrison of Liverpool University The donors of these sera were both Rh positive mothers of erythroblastotic children (table 2) The 2 sera were together described as KJ [3] Miss Boorman and Miss Dodd had found that their serum failed to agglutinate all Rh negatives and this was confirmed with very rare exceptions but I found also that KJ failed to agglutinate St negatives again with very occasional exceptions That is to say it did not react with rr blood nor with R_1R_1 blood It must therefore be reacting with some other frequent gene presumably R_2 and the frequency of bloods agglutinated by KJ i e 30 per cent fitted extremely well with the calculated frequency of bloods containing R (The frequency of the gene R_1 could be taken as the square root of the frequency of the R_1R_1 +− group and the frequency of r from the square root of the frequency of the rr −+ group The difference between the sum of these 2 and unity gave the frequency of the gene R and from these gene frequencies the expected frequencies in various genotypes could be calculated)

as I can make out from the publications, Wiener was not using a pure 30 per cent serum but the mixed serum containing the 85 per cent component as well as the 30 per cent As there was more of the latter, Wiener used it diluted, *in which form it* acted as a 30 per cent serum I think K and J must undoubtedly have been the first 2 pure 30 per cent sera from Rh positive mothers to be discovered

The upper section of table 5 illustrates our genetical interpretation of our results, namely that the Rh locus could be occupied by 7 allelomorphs Wiener, similarly, postulated 6 allelomorphs at one locus

TABLE 4 —*The combined use of 4 anti Rh sera*

85%	80%	30%	0%
RR	− R_1R_1	− R_1R_1	+ R_1R_1
		+ R_1R rare	+ R_1R rare
+	R_1R_2	R_1R_2	+ R_1R_2
	+ R R_2 R_2r	+ R R_2 R r	− R_2R & R r
Rr	R_1r	− R_1r	+ R_1r
			− R r rare
− rr	+ rr	− rr	− rr
			+ R′r rare
		+ R″r rare	− R″r rare
Serological groups –	3	6	9
Genes 2	3	5	7

Some of the rarer genotypes are not shown for example R_2R gives the same reactions as R_1R_1 i e + − − +

At this time that is to say at the end of 1943 Professor R A Fisher examined our table of results He noticed that the reactions of the 70 per cent serum and the 80 per cent serum were antithetical [7]* It looks fairly obvious with the genes arranged in this order In the original table the order of the genes was different and the relationship had escaped notice Fisher supposed that the antigens and the genes recognized by these two antibodies were allelomorphic and called them

* Dr Levine had noticed that bloods negative with the 70 per cent serum were positive with the anti Hr serum but of this we in England were not then aware

blood might be wholly due to the r This could be decided however by making use of the fortunate ability of the 80 per cent serum to distinguish between a double and a single dose of the gene it recognized For example if the 80 per cent serum was titrated against R_1r cells representing one gene positive for it the result was as shown in table 3 Cells representing 2 genes positive for the serum for example rr or R_2r gave a stronger reaction $R''r$ cells constantly gave this stronger type of reaction which meant that not only the r but also the R'' were + with the 80 per cent serum So the question mark could be replaced by a +

It was realized that the 70 per cent reacting serum described by Landsteiner and Wiener in 1941 would agglutinate blood representing the gene R_1 and a search was made for such a serum In the autumn of 1943 Professor Cappell of Dundee found a serum giving 70 per cent positive reactions He kindly gave me a supply and it behaved as anticipated[4 5] (table 4) That is to say it agglutinated all R_1R_1bloods and practically all the ++− bloods which were thought to be R_1r A few were negative however and a further gene R had to be postulated to account for this

TABLE 3 —*The reaction of R″ and the 80% serum*

	Antise a		
	85%	80%	30%
Blood R″r reacted	−	+	+
and as the gene r reacts	−	+	−
the gene R must react	−	?	+

	Titrat on of the 80% (St) Serum					
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cells R_1r	++	+	+	−	−	−
Cells rr	++	++	++	++	+	−
cells R r	++	++	++	++	+	−

It also agglutinated just the right proportion of bloods belonging to the +++ group to account for the R_1R_2 among them It failed to agglutinate the great majority of rr bloods A few were agglutinated however and another fresh gene had to be postulated this gene is now called R

The 4 sera defined 9 serological types of blood and could theoretically define 2 more rare combinations which I did not come across at that time that is to say $R'R''$ −+++ and $R'R$ −−−+ The 4 sera also defined 7 genes of which 26 re actions were determined (table 5) At this time Wiener published the result of his work with 3 of the sera[6] Wiener could define 6 genes and 18 interactions They are here shown in the same form as our results It will be seen that there was com plete agreement between the 2 schemes where they overlapped Lacking the 80 per cent serum Wiener could recognize 8 of the 11 Rh types of blood which we could recognize It should be said that at this time both Levine and Wiener had recognized the 2 mixed sera 85+30 and 85+70 but these 2 sera give no help in isolating genes or Rh types beyond that given by the 30 per cent and 70 per cent sera alone As far

be identified in the 7 (as we had supposed) allelomorphic genes but the eighth combination R_y or CdE has yet to be found and there is a theoretical reason to be mentioned later why it may be very rare Reactions outside the enclosure are those predicted by Fisher, as they are verified serologically parentheses are removed

The first small confirmation of this brilliant theory came when the reaction of the gene R with the 70 per cent serum was found to be positive[8] The positive reaction with the 85 per cent serum did not give any evidence for had it been negative we would have said it was R_1 that we had found But with the 70 per cent serum it had to be positive Dr Murray of the Middlesex Hospital, London, sent specimens of blood from the family of the second R, person I had found (table 7) The man was R_1R the only combination in which R could then be recognized serologically the reactions of which are shown in table 7 As the 85 per cent (anti D) and 70 per cent (anti-C) sera do not give a clear dosage effect like the 80 per cent serum one could not say whether the R_1R blood was positive with these 2 sera only on account of its R_1 or not This man had been married twice

TABLE 6 —*Serological confirmation of Fisher s synthesis*

English positive	Antibodies	Genes and antigenes							
		R CDe	R_1 cDE	r cde	R cDe	R cdE	R Cde	R CDE	(R_y)(CdE)
70	anti C	+	−	−	−	−	+	+	(+)
85	anti D	+	+	−	+	−	−	+	(−)
30	anti E	−	+	−	−	+	−	+	(+)
80	anti-c	−	+	+	+	+	−	−	(−)
65	anti d	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(+)
96	anti-e	+	−	+	+	−	+	−	(−)

The reactions within the enclosure are those known before Fisher formulated his theory The predictions made by the theory are shown outside the enclosure thos yet to be confirmed serologically are shown in parentheses

and the 2 wives were fortunately co-operative The children by the first wife merely confirmed the genotype of the father but the second wife was Rh negative so the child could only be R_1r or R r R_1r reacts ++−+ and the child s blood reacted ++++ so it must be R r and this fixed as positive the reaction of R with the first and last sera That is the 85 per cent or anti D and the 70 per cent or anti C for the + reactions could not be due to the r for this is negative with both sera

Much stronger confirmation came when Dr Mourant who was then working at the N E London Blood Supply Depot found the anti e serum of prophesy[9] and was able to confirm all the reactions expected of it except of course that with R_y which has not yet been isolated This antibody was made by a man suffering from acquired acholuric jaundice He had been repeatedly transfused the transfusions being followed by increasingly severe reactions and it was for this reason that his serum was sent to Dr Mourant The patient was R_2R or cDE/cDE and had been given transfusions of blood from 40 donors most of which would contain the e antigen for only 2–3 per cent of Europeans are EE The most important practical

C and c The reactions of the remaining 2 sera, the 85 per cent and the 30 per cent were not antithetical nor did they bear any relation to the other two sera so to *the antigens that they were recognizing Fisher gave separate loci D and E* Presumably D had an allelomorph for blood is frequently not agglutinated by anti D that is to say it has not D but it must have some antigen that could not then be recognized and this was called d Similarly E was given a hypothetical allelomorph e Fisher supposed that these two hypothetical genes or antigens d and e

TABLE 5 —*Rh antigens and antibodies 1943*

Rh chromosome

R_1 R_2 r	multiple
R R″ R	allelomorphs
or R	at one locus

7 genes 4 antisera 26 interactions (Race Taylor Cappell and McFarland[15]*)*

		R	R_2	r	R	R	R	R_z
	70%	+	−	−	−	−	+	?
antisera	85%	+	+	−	+	−	−	?
	30%	−	+	−	−	+	−	+
	80%	−	+	+	+	+	−	−

6 genes 3 antisera 18 interactions (Wiener[8])

		R_1	R	r	R	R	R
	70%	+	−	−	−	−	+
antisera	85%	+	+	−	+	−	−
	30%	−	+	−	−	+	−

Fisher s interpretation

antibodies	*Rh* chromosome genes and antigens		antibodies
anti C	C	or c	anti c
anti D	D	or (d)	(anti d)
anti E	E	or (e)	(anti e)

were each capable in favorable circumstances of stimulating their own antibodies *like the other antigens and the reactions expected of the two hypothetical anti* bodies anti d and anti e could be tabulated as will be shown in table 6

Fisher supposed that the 3 genes *if genetically separable must be close together* on the chromosome for at the time I am speaking of I had tested over 50 families with the 4 sera and no example of crossing over had been found (I have now tested over 250 families without detecting crossing over)

If this then was the genetic situation an Rh chromosome could be assembled in 8 different ways CDE CDe Cde etc (table 6) Seven of these assemblages could

be identified in the 7 (as we had supposed) allelomorphic genes, but the eighth combination R or CdE has yet to be found, and there is a theoretical reason to be mentioned later why it may be very rare Reactions outside the enclosure are those predicted by Fisher as they are verified serologically parentheses are removed

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70	anti C	+	−	−	−	−	+	+	(+)
85	anti D	+	+	−	+	−	−	+	(−)
30	anti E	−	+	−	−	+	−	+	(+)
80	anti-c	−	+	+	+	+	−	−	(−)
65	anti d	(−)	(−)	(+)	(−)	(+)	(+)	(−)	(+)
96	anti e	+	−	+	+	−	+	−	(−)

The reactions within the enclosure are those known before Fisher formulated his theory The predictions made by the theory are shown outside the enclosure thos yet to be confirmed serologically are shown in parentheses

and the 2 wives were fortunately co-operative The children by the first wife merely confirmed the genotype of the father but the second wife was Rh negative so the child could only be R_1r or R r R_1r reacts ++−+ and the child s blood reacted ++++ so it must be R r and this fixed as positive the reaction of R with the first and last sera That is the 85 per cent or anti D and the 70 per cent or anti C for the + reactions could not be due to the r for this is negative with both sera

Much stronger confirmation came when Dr Mourant who was then working at the N E London Blood Supply Depot found the anti e serum of prophesy [9] and was able to confirm all the reactions expected of it except of course that with R_y which has not yet been isolated This antibody was made by a man suffering from acquired acholuric jaundice He had been repeatedly transfused the transfusions being followed by increasingly severe reactions and it was for this reason that his serum was sent to Dr Mourant The patient was R R_2 or cDE/cDE and had been given transfusions of blood from 40 donors most of which would contain the e antigen for only 2–3 per cent of Europeans are EE The most important practical

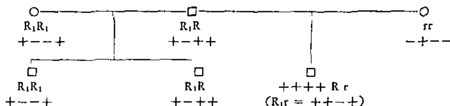
advance made by this serum is the distinction between R_2r cDE/cde and R R_2 cDE/cDE blood

Finally Dr Diamond has found the anti d antibody (Some more of the paren theses in tables 6 & 14 could therefore be removed)

Table 8 shows the chromosome frequencies calculated by Fisher by a maximum likelihood method from the first 927 unselected bloods that I tested with the 4 sera [10] The R″ in this series was undoubtedly by chance too high It should be nearer 0 8 Fisher noticed that the chromosomes could be arranged in 3 orders of frequency 1 over 12 per cent another round about 1 per cent and a third so rare that it has not yet been isolated He made the fascinating suggestion that if one considered that these 3 frequent chromosomes were the basic European combina tions then the heterozygotes that is to say R_1r R_1R_2 and R_2r could give on crossing over R′ and R_o, R and R R″ and R_o which have the second order of frequency However they could not give R_y CdE which would require a double

TABLE 7 —*The reaction of the gene R with the 70% and 85% sera*

	Anti e a			
	85%	80%	30%	0%
Blood R_1R	+	−	+	+
but gene R_1	+	−	−	+
gene R	?	−	+	?
gene r	−	+	−	−



crossover Moreover on this theory R would be expected to occur with a fre quency about equal to that of R′ R″ and R put together which is just about what is found

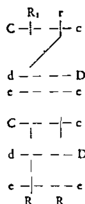
Wiener[11] has attacked this idea on the ground that frequencies in other races do not fit and he gives as an example those of American Negroes where the gene R_o is 42 per cent far higher than the sum of R′ R′ and R Wiener has missed the point that while the idea is capable of explaining the less frequent combinations in a population it was never supposed to explain the more frequent ones To test this theory one must accept the observed frequent combinations in a race and then see what their heterozygotes would give on crossing over The few adequate racial studies so far reported fit the theory remarkably well Wiener s American Negroes among them As an example in Wiener s 95 Mexican Indians [1] the only frequent genes or chromosomes were R_1 and R_2 So the only frequent heterozygote would be R_1R which would give on crossing over an R and an R_o chromosome Ac cording to the theory one would expect then about equal gene frequencies of R

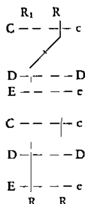
and R in this population In the small sample of 95 bloods the frequencies were R_0 1 9 and R 3 3

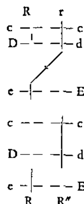
Fisher has carried the theory a step further [10] According to this idea R″ represents a crossover between D and E R one between C and E and R′ one between C and D Now the ratio of the frequency of the chromosome R″ to the frequency of its parent heterozygote R r is much higher than that of R to R_1R_2 or R′ to R_1r and this suggests that the distance on the chromosome between D and E is greater than that between C and E or between C and D Fisher therefore considers the order is probably DCE A series of English bloods that we are at present testing is fitting in extraordinarily well with this order Wiener[11] has attacked this idea on the grounds that the true frequency of R″ is about the same as R′ and not as high as I found in the 927 bloods This is probably quite correct, and indeed we said so in the paper[10] which Wiener was attacking but it makes no difference to the argument

TABLE 8 —*Rh chromosome frequencies in England* (from *Fisher* and *Race* 1946)

R_1 CDe	43 61%	R″ cdE	1 70%
r cde	37 90%	R Cde	0 81%
R_2 cDE	12 80%	R CDE	0 13%
R cDe	3 05%	(R_y)CdE	0 00%







If we allow say 1 per cent to R′ and to R″ then the ratio R″ to R r is 1 to 10 still very much higher than R′ to R_1r which is 1 to 33

Recently Dr Sheila Callender and I have found a third allelomorph at the C c locus[13] which we call C^w A patient at the Radcliffe Infirmary Oxford needed numerous transfusions The patient was of the genotype CDe/CDe (R_1R_1) and as she had already made anti c she required blood of her own genotype and this we thought she was given However a new antibody appeared in her serum following this transfusion which agglutinated the blood of the donor This serum we tested against a large number of bloods of known genotype with the results shown in table 9 This suggested that the antigen recognized by this antibody was connected with C for bloods containing 2 C were perhaps twice as often positive as bloods containing 1 C (or at least the observed figures did not contradict such a hypothesis) and bloods containing no C were constantly negative This suggested that an occasional gene that we were classifying as C was really different and we called it C^w The only reason for choosing W was that the donor s name was Willis

The family shown in table 10 confirmed our ideas The circles represent females the squares males Black indicates that the new antigen was present, the hollow that it was absent In this family the mating was happily such that it is possible *to say exactly which of her Rh chromosomes the grandmother had given to each* of her children It will be seen that when she handed on her R_1 (which contains C) she also handed on her new antigen When an R_1 is underlined it means that it is unequivocally grandmaternal To 5 of her children she gave an R (which does not contain C) but not the new antigen to 4 she gave R_1 and the new antigen *If this coincidence of R_1 and the new antigen was only accidental and due to chance* then the probability of getting the distribution observed in this one generation is only 1 in 126 One child in the next generation can be added to this calculation and the probability is reduced to 1 in 252 That is to say if we suppose the coincidence of R_1 and the new antigen is merely accidental then we would only expect to get such a perfect fit as this once in 252 such families

This and other families made it quite certain that the new antigen was another form of C rather a rare form It could not be another form of D or e (the other components of R_1) because all R r people who contain D and d, E and e lacked the new antigen

TABLE 9 —*Reactions of the new Rh antibody* (from *Callender* and *Race* 1946)

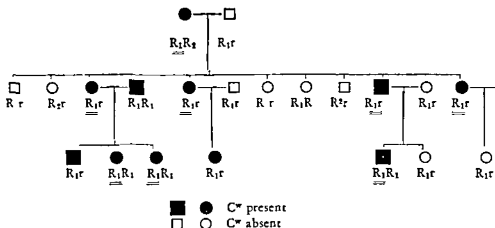
	+	−	Total	Positive
CC	12	193	205	5 9%
Cc	7	342	349	2 0%
cc	0	214	214	0 0%

Up to this stage in the investigations all the C^w that had been found were in the combination C^wDe (table 11) I supposed that it must exist in the combination C^wde and with a frequency just great enough to make it worth looking for The combinations C^wDE and C^wdE were presumably possible but might be excessively rare After testing 1500 bloods kindly sent by Dr W S Stanbury the combination C^wde was found rather sooner than I had dared to hope As this blood was such a rare find I tested it with all the 70 per cent sera that I could muster and unexpectedly some were + and others − This made it clear that some of the sera called anti C 70 per cent or anti Rh were really mixtures of anti C + anti C^w, while others less than half of all were pure anti C The sera I had been using in the investigation up to this time were of the mixed type anti C + C^w C^w had therefore appeared to be something split off C for C^w people always appeared to have C as well whether they really had it or not Actually C^w was an alternative to C (and therefore to c)

With the 3 sera anti c anti C^w and the newly recognized pure anti C a good deal of light could be focussed on this locus with its 3 allelomorphs (table 12) It appeared on paper that the rare homozygote C C^w would be recognizable if it turned up but owing to the low frequency in the general population (1 in 10 000) it was clearly useless to seek for it in random bloods but there was a more hopeful

line of search There was a mating $C^w \times C^w$ (table 13) shown as part of the large pedigree, already seen in table 10 As far as they knew the husband and wife were not related All 5 people had been tested with anti C^w, and found to be positive but before the recognition of the two kinds of 70 per cent sera, which made the identification of a homozygote possible There was no chance of this boy being homozygous C^wC^w because he had c from his mother, but there was a 50 50 chance of one or the other daughter being homozygous Fresh samples of blood were obtained and to our great satisfaction one daughter was homozygous and the other heterozygous the homozygote giving the anticipated characteristic reaction This was confirmed in an unexpected but very nice way, for the cells of the homozygous

TABLE 10—*The antigen* C^w (from *Callender* and *Race* 1946)



The underlined R_1 are unequivocally grandmaternal

The genotypes shown are those which would have been given to the blood before the elucidation of the relationship of the new antigen (Willis) to C R_1r Willis positives for example are now known to be C^wde/cde and not CDe/cde and would not now be called R_1r The anti Rh (70%) serum used in making the diagnoses of genotypes shown above was of the type now recognized as being anti C + C^w

sister were agglutinated by much higher dilutions of the anti C^w serum than were the cells of the heterozygous sister and other heterozygotes

Genetically the relationship between C c and C^w is clear they are allelomorphs But antigenically all is not yet clear for C can stimulate anti C + C^w which was not like the behavior of any other Rh antigens known up to last year for they all behaved simply e g E stimulated anti E and nothing else c anti c and nothing else etc Moreover this natural mixture of anti C + C^w cannot be split by absorption with C or with C^w cells (An artificial mixture made by adding a pure anti C to the pure anti C^w can easily be split by absorption) Antigenically then C^w seems to have something in common with C But the dosage effect of anti C^w lines it up with c which also shows this effect strongly anti C like anti D and anti E gives only a slight dosage effect which is difficult to demonstrate

If Fisher had not discovered the allelomorphism of C and c and of D and d and

The family shown in table 10 confirmed our ideas The circles represent females the squares males Black indicates that the new antigen was present the hollow that it was absent In this family the mating was happily such that it is possible to say exactly which of her Rh chromosomes the grandmother had given to each of her children It will be seen that when she handed on her R_1 (which contains C) she also handed on her new antigen When an R_1 is underlined it means that it is unequivocally grandmaternal To 5 of her children she gave an R_2 (which does not contain C) but not the new antigen to 4 she gave R_1 and the new antigen If this coincidence of R_1 and the new antigen was only accidental and due to chance, then the probability of getting the distribution observed in this one generation is only 1 in 126 One child in the next generation can be added to this calculation and the probability is reduced to 1 in 252 That is to say if we suppose the coincidence of R_1 and the new antigen is merely accidental then we would only expect to get such a perfect fit as this once in 252 such families

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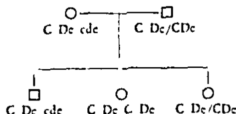
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lelomorph at the D locus,[18] which he called D (table 15) It was found in the combination cD E/cde It looks as if D' will be a close parallel to C* although the *investigation is very much limited by the lack of a pure anti D' serum and by the*

TABLE 13 —*A C Iemw...* (*from Callender and Race*)



Blood of	Anti—					
	C	D	E	c(D)	e	c
1st child	−	+	−	+	+	+
2nd child	−	+	−	−	+	+
3rd child	+	+	−	−	+	+

lack of anti-d Stratton recognized D when he tested these cells with several good anti D sera Some reacted strongly some weakly and some not at all That is to say many anti D 85 per cent sera are really anti D + D , while some are pure anti D

TABLE 14 —*Rh antigens and antibodies* (from *Race Mourant* and *Callender* 1946)

Antibodies	Genes and antigenes											
	R cDe	r cde	R_2 cDE	R cdE	R CDe	R Cde	R CDE	R_y CdE	C De	C*de	C*DE	C*dE
Anti C	−	−	−	−	+	+	+	(+	−	−	(−)	(−)
Anti D	+	−	+	−	+	−	+	(−	+	−	(+)	(−)
Anti E	−	−	+	+	−	−	+	(+)	−	−	(+)	(+)
Anti c	+	+	+	+	−	−	−	(−	−	−	(−)	(−)
Anti d	(−)	(+	(−)	(+)	(−)	(+)	(−)	(+	(−)	(+)	(−)	(+)
Anti e	+	+	−	−	+	+	−	(−)	+	+	(−)	(−)
Anti C*	−	−	−	−	−	−	−	(−)	+	+	(+)	(+)

The left upper compartment shows the interactions known before *Fisher's* theory was postulated The middle compartment shows the extension demanded by *Fisher's* hypothesis now in part confirmed serologically The right and lower compartment shows the extensions made and those suggested by the anti C* serum Reactions which have not yet been confirmed serologically are shown in parentheses In this table anti C means pure anti C and not anti C + anti C* which is the constitution of about half the anti Rh sera

just as in the case of anti C + C and anti C But there is yet no pure anti D like the pure anti C* Absorption of the mixture has failed to split the two components Stratton has collected the family of his D donor and it shows the expected inheritance of the allele He kindly sent me some blood from this donor and I was able

of E and e C^w would have presented a problem that we at any rate could not have solved

The table of interactions between Rh antigens and antibodies must now be ex tended thus (table 14) The left upper compartment shows our results before Fish er s theory was postulated the middle compartment shows the extension demanded

TABLE 11

C^{w}De	C^{w}de	C^{w}DE ☐ C^{w}dE
many found 1% of European chromosomes	one found	not found but presumably possible

	Anti					
	D 85	r 80	F 30	C 0	C^w 2	c 98
C de/cde	−	+	−	±*	+	+

+ with some 70% sera (anti C + C^w) − with other 70% sera (anti C)

by Fisher s hypothesis now in part confirmed serologically the confirmation being shown by removal of parentheses The right and lower compartment shows the ex tensions made and those suggested by the anti C^w serum Anti C in this table means pure anti C and not anti C + C^w which is the constitution of more than half of the 70 per cent sera

The 9 known Rh chromosomes make 45 possible Rh genotypes but there are only 24 serologically distinguishable Rh groups using the 6 sera anti C c C^w

TABLE 12 —*Reactions and f equency in Englan l of the possible c nb nations f C C^w and c* (*Call n ler* and *Ra e*)

	Ant	Anti C (pure)	Ant C^w	
C C^w	−	−	+	0 01%
C C	−	+	+	0 94%
CC	−	+	−	18 40%
C c	+	−	+	1 23%
Cc	+	+	−	48 05%
cc	+	−	−	31 36%
				99 99%

D E and e Some of the reaction groups contain only 1 genotype others contain 2 or 3 but as one member of the group usually is so very much more frequent than the rest one can make a pretty good guess at the actual genotype of the blood being examined

This was the end of the story except for the genes called by Wiener intermedi ate In February of this year Dr Stratton of Manchester identified a third al

in the chromosome R_1 (CDe) If such names as Wiener s Rh_0[12] are used one would have to speak of D as the o component and C as the prime component This is possible though clumsy but to refer to the c component is impossible

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[14] Race R R Mourant A E and Callender Sheila T Nature *15* 410 1046
[15] Stratton F Nature *158* 25 1946

DISCUSSION

Dr Urbe My friends I have a paper here from Dr Robert Russell Race on the Rh Genotypes and Fisher s Theory Dr Strandskow if you will come to the microphone

Dr Strandskou I feel somewhat like a stranger here I am not a hematologist but supposedly interested a little bit in genetics I can t help but admire the very splendid work being done by hematologists such as Dr Levine Dr Race and others which has stimulated the genetics field within the last few years It has been an inspiration to those of us who are more interested in straight genetics

I don t know if I have any particular comment except perhaps to say that the 3 loci idea is not too common to geneticists I am not sure that it is much more common than the other illustration that you had Dr Race It is certainly not the usual thing that you find It is a little bit difficult to imagine 3 loci very close together on a chromosome affecting the same character so to speak I know of no other similar illustration among the lower forms not even in man and certainly not in such forms as Drosophila where we have perhaps 15 or 20 loci affecting eye color In such instances none of the loci are close together they are distributed throughout all the chromosomes and I think that s what one might expect There is perhaps one exception and that is the Muller situation (I think some of you are familiar with it) where there is some indication that we had as a result of a very abnormal type of crossing over between two loci but to get three loci very close together giving crossover results is indeed an unusual thing and it would surprise me very much if it turns out to be true I would be very much interested in it

I have not made any decisions I had hoped to have a further conversation with Dr Race and perhaps clear up some questions as to which theory I favor at the present time I am certainly inclined toward the recent presentation but I want to be very cautious and I should like to urge everyone else to do so We can t afford to have another 4 or 5 years of the confusion that we have had the last 4 or 5 years so I hope that the evidence will be a little better than what we have had in the past for some of the theories advanced I am sure Dr Race feels the same way and he has been very cautious I don t believe I have any more comments

I would like to ask a question of Dr Race and it pertains in particular to the double crossovers Is th percentage of double crossovers that you had equal to the product of the two single crossovers? That of course you should expect theoretically in the population That s always true with respect to the double crossover that they can never be larger than either of the single crossovers and theoretically should be equal to the product of the two single crossovers I am also int r sted as to what explanation you might have for the fact that the original chromosome alignment was not CDE but some other arrangement so that your crossover represents the coming together of the CDE representing the crossover results

to classify my anti D sera and to find several examples of D^{u} p-ople and also one of the other possible chromosome combinations CD e both in the genotype CD e/cde and CD^{u}e/Cde which latter must be a very rare blood

Stratton considers that D is Wiener s intermediate gene and this seems very probable and we welcome it to Fisher s scheme especially because Wiener has used the intermediate gene as an argument against Fisher s ideas in general

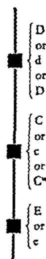
TABLE 15 —*The D allelomorph (Stratton)*

	D 85	c 80	E 30	C 70	e 98	C^{w} 2
cells cD E/cde	±*	+	+	−	+	−

* + with some 85% sera (= anti D + D) − with other 85% sera (= anti D)
Other combinations found CD e/cde CD e/Cde

The antigenic allelomorphisms revealed by Fisher are fundamental to an understanding of the Rh blood groups When Fisher s ideas were put forward they brought order and essential simplicity to a confused mass of apparently arbitrary facts about Rh antigens and antibodies They made certain predictions most of

TABLE 16 —*The Rh chromosome according to Fisher showing how C^{w} and D fit in*



which have since been verified serologically and they enabled some new discoveries to be successfully interpreted Fisher considers that the genetic basis of these allelomorphic antigens is a system of 3 closely linked genes (table 16) and has produced some indirect evidence that crossing over may have occurred

Note

I must apologize for using the CDE notation with which you are probably not familiar but in a serious discussion on the Rh genes and antigens it is at present the only possible one One needs to be able to speak of the C and D component

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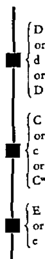
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The antigenic allelomorphisms revealed by Fisher are fundamental to an understanding of the Rh blood groups When Fisher's ideas were put forward they brought order and essential simplicity to a confused mass of apparently arbitrary facts about Rh antigens and antibodies They made certain predictions most of

TABLE 16 —*The Rh chromosome according to Fisher showing how C^{w} and D fit in*



which have since been verified serologically and they enabled some new discoveries to be successfully interpreted Fisher considers that the genetic basis of these allelomorphic antigens is a system of 3 closely linked genes (table 16) and has produced some indirect evidence that crossing over may have occurred

Note

I must apologize for using the CDE notation with which you are probably not familiar but in a serious discussion on the Rh genes and antigens it is at present the only possible one One needs to be able to speak of the C and D component

HEMOLYTIC MECHANISMS

By WILLIAM DAMESHEK M D

ERYTHROBLASTOSIS foetalis is a violent acute hemolytic anemia occurring during the very first few days of life It represents an example of acute hemolytic anemia occurring congenitally and due to the passive transfer from the mother to the fetus of antibodies which injure the fetal red cells As an acute hemolytic anemia this disorder is subject to the same physiopathologic mechanisms as are found in other types of hemolytic anemia and I will discuss hemolytic mechanisms in general with the idea of presenting a background for the proper interpretation of hemolytic disease of the newborn

Physiologic principles The exact mechanisms involved in normal red blood cell destruction are quite obscure It is known that the mature non nucleated erythrocyte has a life span of approximately 110–120 days from the time of its delivery from the bone marrow to the circulation [1] In this period of time the cell passes through many miles of capillaries and is subjected to much squeezing and bumping participates in many thousands of chemical exchanges in both the lungs and the tissue and may remain stagnant for hours at a time in the spleen and in other sinusoidal organs Despite the cell s plasticity and almost complete lack of its own metabolism (it is without a nucleus) it inevitably wears out Does it then simply become lysed by such normally present metabolites as lysolecithin or lecithin? Does it become fragmented or does it become swollen and disintegrate in the so-called graveyard of the red cell—the spleen? Answers to these questions are as yet not fully available Peculiarly enough more is known of abnormal blood destruction than of normal

The course of the pigment hemoglobin is fairly well worked out but the fate of the cell itself and of its many other constituents is rather obscure The normal red cell a biconcave disc with an average volume of 85–95 cu micra and an average diameter of 7 5 micra has an average thickness of 2 0 micra This cell when placed in normal or isotonic salt solution (0 9 per cent NaCl) remains essentially unmodified However when placed in solutions of progressive hypotonicity—(0 8 per cent 0 7 per cent 0 65 per cent etc) the cell takes on more and more fluid In a solution of 0 6 per cent NaCl the red cell is thicker (with fluid) than in a solution of 0 8 per cent NaCl Even though thicker however its total volume remains essentially unchanged In other words as the cell becomes thicker it becomes rounder more spherical and smaller in diameter In 0 5 per cent NaCl solution the red cell is almost completely spherical although its volume is still 85–95 cu micra its diameter may now be only 4–5 micra and its thickness almost the same amount In solutions of NaCl under 0 5 per cent the red cell finally bursts (hemolyzes) The differences in initial hemolysis indicate a difference in the thickness of the red cell population The more resistant, i e the thinner red cells are probably for the most part the younger ones (reticulocytes) which have only recently been delivered from the

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rather than the original results That I think is a rath r interesting situation and I think it would be of interest to all of us to have that relationship explained if you can

Dr Race *The frequency of the* R_y *is quit unknown b caus it hasn t yet b-en obs rv d at all* but it must b- less than the products of th crossovers

Dr Levine This question is very important from th point of view of human g n tics We know re markably little about human transmittable prop-rties and it so happens that th normal variations we know most about are the blood factors The probl m is very difficult to attack because som of the rare sera are not readily available and we cannot make comprehensive studi-s of families rac s and different populations By the application of gene frequencies and the heredity studies it should be possible to com to a decision in a comparatively short time Until we have these rare sera I am afraid we will not be able to have all the complete evidence we would like to have in support of the Fisher theory Whether or not the geneticist is accustomed to linkage with multiple allelomorphisms of course is another matter We do not know too much about the human chromosomes and the loci The only thing I d like to tell Dr Strandskov is how terribly surprised the geneticist was when I first described the selective fetal death not due to homozygous lethal factor and the effect on a heterozygous individual Now we have a very practical point to consider here and of cours- that is a matter of the terminology What are we going to do about terminology? I should like to suggest that the Fisher theory takes into account a very important element and that is the Hr sera It is a great tribute to Dr Fisher that his theory takes these into account It is remarkable that he not only predicted d and e but calculated the incidence of their occurrence and per cent specificity of the sera against these Hr antigens So whether a gen ticist is or is not accustomed to the idea of multiple linkage I being only a hybrid geneticist would tend very much to accept the Fisher theory But th terminology is of course the crux of th situation What are we p-ople going to do about the terminology? Well I think w must get accustom d to think in terms of thes 3 Rh sera and we don t have to worry about th- Hr sera until we g t a large enough supply I think that for the time being one has to use both terminologies A change in terminology is essential I have been using the Fisher terminology and I think it simplifi s matters to a consid rable degree but for th time being we will probably have to use both until we gather family results and study the races whi h will I hope on the basis of gene frequencies confirm Dr Fisher s theory

Dr Race I agree with Dr Levine I don t think it s possible to think of any 1 locus to bring in these known allelomorphs without postulating sub-genes allelomorphi sub-genes I think it best if we can use the CDE because that doesn t commit us to any chromosome arrangement but it does describe exactly what we find serologically

zone i e spherocytes They are unusually fragile in hypotonic solutions of sodium chloride Extreme spherocytosis is the rule in violent hemolysis, whether in the experimental animal or in the hemolytic crisis clinically The simultaneous occurrence of spherocytosis with anemia, and the coincidence of extreme degrees of spherocytosis with severe blood destruction suggest that the spherocyte in its various gradations, from a slightly thickened to an almost completely spherical cell, is the forerunner of complete hemolysis The spherocyte may thus be considered as a red cell which has been injured by a hemolytic agent and as a stage between a normal mature circulating red cell and one which is completely hemolyzed The greater the degree of spherocytosis which is present the more fulminating is the hemolytic process

In less fulminating hemolytic processes in which the red cell count may drop from 5 0 M to 1 5 M in a matter of 3 to 10 days hemoglobinemia and hemoglobinuria do not develop The evidences of hemolytic shock are slight or completely lacking and there is far less strain on the circulation

The anemia is variable and is normochromic normocytic by cell volume, but microcytic by cell diameter i e the red cells are unusually thick and although their diameters are smaller than normal their normal volumes are retained The blood smears show 2 outstanding features which are of physiologic importance (1) a great diversity in red cell population both in size and degree of maturity, and (2) evidence of regenerative activity on the part of red cells white cells and platelets The sudden but not extremely violent hemolysis acts as a powerful stimulant to bone marrow activity, which is reflected in the peripheral blood Thus, together with partially hemolyzed cells i e spherocytes there are present relatively huge red cells newly arrived in the circulation these are polychromatophilic reticulocytes The disproportion between the small spherocyte which is brown and orthochromatic and the large polychromatophilic red cell which is bluish gray in color is quite striking and is readily seen by inspection of a well spread well stained blood smear The 2 cells represent 2 different physiologic processes one destructive the other regenerative The diversity in size of these cells gives rise to 2 types of red cell population with respect to diameters and is well brought out in Price Jones curves of red cell diameters which show a biphasic character

Other indications of increased hemolysis are to be found in the breakdown products of the hemoglobin [4] The plasma bilirubin becomes increased to variable levels depending in part upon the degree of blood destruction and in part upon the functional capacity of the liver to remove excess bilirubin from the circulation Two individuals with the same degree of blood destruction may show bilirubin levels respectively of 2 and 4 mg per 100 cc of blood The presumption is that in the latter case the hepatic cells remove bilirubin from the circulation more slowly than in the first instance With rare exceptions the bilirubinemia is always of the indirect variety and is thus associated with urine which is free of bilirubin i e acholuric jaundice is present Rarely is the amount of bilirubin presented to the hepatic cells so high and the cells simultaneously inefficient (as in hemolytic anemia of the newborn) that a mixed type of bilirubinemia with bile appearing in the urine is present The total amount of bilirubin in plasma rarely exceeds 10

marrow the thicker ones are probably the older cells which have been buffeted about in the circulation and have remained stagnant in various sinusoidal areas The thinnest cells do not become completely hemolyzed until concentrations of approximately 0 25 per cent NaCl are reached Thus some normal red cells are hemolyzed at approximately 50 per cent NaCl solution and all are hemolyzed at approximately 25 per cent NaCl The red cell as Guest[2] has pointed out may be considered a perfect osmometer responding quickly to changes in hypotonicity and thus showing quick changes in thickness These changes of the red cell with respect to hypotonicity are made use of in the fragility test But they may have some physiologic importance as well That is the more effete red cells may be smaller and thicker than the relatively immature cells just released from the bone marrow Although the question of hypotonicity does not enter into the ultimate hemolysis of the thickest red cells within the circulation it is highly probable that these cells are more vulnerable to breakdown—say within the spleen—than their thinner fellows The spleen may well be the graveyard of the thickest red cells

Pathologic physiology of increased red cell destruction The sequence of events which takes place during a bout of sudden blood destruction is best studied in the experimental animal Guinea pigs when injected with an anti red cell hemolytic serum (see below) *develop either fulminating acute or subacute hemolytic processes depending* upon the amount of hemolytic serum injected [3] In the fulminating type there is hemoglobinuria very rapidly developing anemia extreme spherocytosis of the red cells Evidences of regenerative activity on the part of the marrow are lacking The hemoglobinuria and the violent reduction in red cell count (from about 5 0 M to 1 0 M or thereabouts in 24–36 hours) are indications of blood destruction which is so rapid that liberated hemoglobin cannot be modified to bilirubin but is excreted from the blood plasma into the urine

Hemoglobin in the plasma is a threshold substance it is present normally in a concentration of approximately 5 mg per 100 cc When the threshold of approximately 150 mg is exceeded hemoglobin is excreted into the urine Hemoglobinuria thus indicates rapid and violent blood destruction which occurs usually within the circulation i e intravascularly Hemoglobinuria is always accompanied by hemoglobinemia but very definite hemoglobinemia (up to 150 mg per 100 cc) may be present without hemoglobinuria

In fulminating hemolysis the red cells in the circulation may become decreased by 60 per cent or more within a matter of a day or less This may lead to a variety of shock hemolytic shock due to the sudden loss of an *osmotically* important substance from the circulation When one considers the great loss in total red cell volume from approximately 2250 cc in a normal human adult to (say) 450 cc it is readily seen that the entire body must adjust itself quickly or death will supervene The great strain on the circulation more particularly on the heart and on the tissue cells in a variety of organs can only be speculated upon

The red cells which remain within the circulation after an attack by a hemolytic agent are presumably those which were originally the most resistant Inspection of a blood smear at this point reveals that even these cells are now almost exclusively small usually round dense brown staining and devoid of a central clear

loss of this hemolytic ability, but when fresh guinea pig serum ("complement") was then added hemolysis took place

It was determined that hemolysis was a 2 step affair (1) sensitization by antibody or amboceptor and (2) actual hemolysis by another substance complement These observations although well known for the most part by immunologists were little if at all appreciated from their clinical standpoint The first hemolysin which was related to clinical disease was that described by Donath and Landsteiner in cases of paroxysmal cold hemoglobinuria These investigators demonstrated that certain, usually syphilitic, individuals had an autohemolysin in their sera which reacted with chilling and then reheating The French observers Chauffard Troisier Widal, Abrami and Brule, and others demonstrated hemolysins in some of their cases of hemolytic anemia, some of which they called hemolysinic anemia [6] They believed that these hemolysins, which acted upon the patient s own red cells (auto hemolysins) were causally related to the hemolytic process present

Chauffard[8] believed that a new speciality immuno hematology might well be introduced These observations, made between 1910–1915 were apparently disrupted by World War I and then frequently forgotten (or ignored) so that in publications during the next two decades or more there was little mention of the possible role of hemolysins Schwartz and I, in 1937[9] described 3 cases of acute hemolytic anemia in 2 of which hemolysins of the immune body type were definitely present The serum of these cases hemolyzed not only the red cells of many prospective donors but also the patient s own red cells This hemolysis could be prevented by heating the serum to 56° C but reappeared when guinea pig serum (complement) was added The improvement of these patients following splenectomy together with the cessation of spherocytosis and of increased hypotonic fragility *in association with the disappearance of hemolysin* led to the concept that the immune hemolysin might be responsible not only for the hemolytic state but for the spherocytosis and increased hypotonic fragility as well This was directly contrary to the concept of the numerous authorities that spherocytosis was due to a disorder of bone marrow erythrocyte production and not to the activity of such extrinsic agents as hemolysins

An immune hemolytic serum was produced by injecting guinea pig red cells repeatedly in rabbits The rabbit serum was then injected in varying doses in normal guinea pigs and various types of hemolytic anemia were produced *fulminating* with extreme spherocytosis hemoglobinuria and death without regenerative activity on the part of the bone marrow *acute* with spherocytosis and marked changes in fragility but without hemoglobinuria and with evidences of regeneration *subacute* with marked regeneration many reticulocytes and polychromatophilic red cells giving a pseudo macrocytic type of blood picture etc These observations demonstrated (1) that a hemolytic serum could produce hemolytic syndromes in vivo comparable to clinical hemolytic syndromes (2) that spherocytosis was a precursor of hemolysis and could be acquired and (3) that spherocytosis was produced outside the bone marrow since the young red cells in the circulation were much larger than the spherocytes From these and other observa

mg per 100 cc and is usually between 2 and 5 mg The biliary canaliculi are congested with bilirubin and the gall bladder is called upon to store unusually large quantities of bile laden with high concentrations of that pigment As a result bilirubin may precipitate out and form stones with or without a calcium matrix

The intestinal tract receives unusually large quantities of the pigment [5] In violent hemolysis actual discharge of bile may occur in less severe cases the stools are highly colored but otherwise normal in appearance Except in certain rare instances of completely intravascular hemolysis the urobilinogen in the feces is by far the surest index of increased blood destruction It is conceivable in a given case that the liver may be functionally so adequate that it removes newly formed bilirubin almost as soon as it enters the blood stream the excess bilirubin is however passed into the intestine where it is converted into urobilinogen In acute hemolytic anemia the output of urobilinogen may be increased 5 to 20 fold In estimating the degree of hemolysis one should always relate it to the hemoglobin content and even more accurately to the total intravascular hemoglobin Thus an output of 100 mg of fecal urobilinogen may be normal for an adult weighing 150 pounds and with 15 0 Gm of hemoglobin per 100 cc For an adult with 5 0 Gm of hemoglobin however 150 mg of urobilinogen represents an approximately 3 times normal blood destruction for a child weighing only 50 pounds the same amount of urobilinogen at a similar hemoglobin level is about 9 times normal The most accurate estimation of the degree of blood destruction is made by having recourse to the hemolytic index which depends upon knowledge of the blood volume the hemoglobin concentration and the output of urobilinogen in the feces [5]

With an increase in urobilinogen in the intestines the output in the urine usually becomes increased The degree of urobilinogen is also conditioned to some extent by the hepatic function thus if the liver is normal the urobilinogen may pass through quickly and re enter the intestines If on the other hand the liver is damaged there is a delay of urobilinogen excretion by this organ and a consequent increase in the general circulation and thus in the kidneys and urine To rely solely on the content of urinary urobilinogen for the degree of blood destruction may lead to error

Pathogenetic mechanisms of increased hemolysis The various mechanisms responsible for increased blood destruction have recently been given some attention They include the activity of hemolysins and agglutinins the passive action of erythrostasis the role of the spleen as well as of such physical factors as cold heat hydrogen ion concentration etc Attempts to incriminate a single mechanism as solely responsible for all hemolytic states are probably unwarranted

Hemolysins The older immunologists including Bordet Ehrlich and others utilized the activity of hemolysins and their action upon red cells in their work on the theoretical aspects of immune processes [6] The Wassermann reaction is a direct outgrowth of this work They demonstrated that if red cells of one species were repeatedly introduced into the circulation of another the serum of the latter would eventually cause hemolysis of the red cells of the former Thus if guinea pig red cells were repeatedly injected in a rabbit the rabbit s serum would eventually cause hemolysis of the guinea pig s red cells Heating the serum to 56°C resulted in a

tempt has been made to divide agglutinins according to these differences into those which are bivalent and those which are univalent Agglutinins acting well in normal salt solution are said to be bivalent or complete antibodies, whereas those which require the use of a serum or plasma factor are said to be univalent or incomplete antibodies The term blocking antibody has also been used for the incomplete anti Rh agglutinin Whether these concepts are correct, or whether the differences in antibody activity relative to salt solution or plasma rely solely on the type of diluent remain questions for further study

Concanavalin A [7] A protein derived from the jack bean causes intense agglutination of many species of red cells in very dilute concentrations Actual injury to the red cell membrane occurs as evidenced by the behavior with mechanical trauma Hemolysis in vivo is probably induced by the mechanical trauma of the active circulation upon the agglutinated corpuscles although undoubtedly other factors such as temperature change, stasis etc , have their effects

Cold hemagglutinin, found in primary atypical pneumonia and at times in high concentration in other conditions, acts much like concanavalin It is universal in its scope (panagglutinin) acting on all types of red cells, human and otherwise, but is limited in its activity with respect to temperature It has a thermal amplitude of 0°C to 17-20°C In this temperature range it causes intense agglutination of red cells Agglutination if continued injures the red cells as evidenced by mechanical hemolysis with trauma The traumatizing effects of the circulatory pulsations upon agglutinated red cells are probably responsible for the in vivo hemolysis

The iso-agglutinins anti A and anti B and the warm agglutinin anti Rh cause intravascular hemolysis when introduced into the circulation in contact with susceptible red cells containing the appropriate agglutinogen Again similar mechanisms are probably operative injury to red cell membrane with the development of spherocytosis the effects of trauma upon agglutinated red cells complete hemolysis either intravascularly or in the spleen with or without the added effects of stasis

Erythrostasis The effects of simple stasis in the development of hemolysis have been known for years Ham and Castle[10] chiefly on the basis of Knisely s interesting histophysiologic studies of the spleen concluded that the normal spleen has 2 main functions both reproducible in the test tube erythrostasis and erythroconcentration From further experimental data they concluded that all hemolysis was a function of erythrostasis either (1) of unusual degree or (2) of normal degree in the presence of abnormal cells i e spherocytes Stasis of unusual degree was often the end result of agglutination Stasis of normal degree with abnormal cells was present in various types of anemia with spherocytosis and occurred chiefly within the spleen

Although there can be no doubt that erythrostasis particularly within the spleen may have some slight effect in the ultimate hemolysis of red cells, this theory does not explain the development of spherocytosis nor does it indicate that the spherocyte is simply a red cell already in the process of hemolysis Furthermore as already indicated above an agglutinating substance does more than simply remove a number of red cells from the active circulation to stagnate and to hemolyze it actually injures the red cell envelope Hemolysis is thus an active rather than a

tions we concluded that the hemolytic syndromes were in all probability more or less concerned with the activity of hemolysins of different types and acting in different concentrations Thus a large dose of hemolysin might result in hemoglobinuria and a smaller dose in subacute or chronic hemolytic anemia

Further observations have borne out these original conceptions with the qualification that by a hemolysin is meant *any* substance chemical, immune or other which directly or indirectly tends to injure the red cell and thus cause its destruction [7] However certain modifications and additions to the original concept must be made

Hemolysins may be classified as simple and complex A simple hemolysin, which may be of chemical nature (saponin lecithin lysolecithin, arsine gas some of the sulfonomides or their end products benzol etc) acts directly on the red cell causing its immediate hemolysis without the intervention of any other substance On the other hand other hemolysins are complex requiring the preliminary action of amboceptor before hemolysis takes place by complement Of the complex hemolysins studied including colloidal silicic acid and immune hetero hemolysin the latter had all the properties of the isohemolysin which we had previously studied and which had earlier been described by Chauffard and Troisier

Complex hemolysin apparently acts first by injuring the red cell membrane as evidenced either by direct observation of the cells which show irregular spinous processes or by the method of mechanical fragility With the latter method shaking of the red cells already acted upon by hemolysin in the absence of complement results in hemolysis within a certain period of time control red cells showing no hemolysis Prompt hemolysis of sensitized red cells takes place when complement is added the latter agent apparently being the actual hemolysin Complete hemolysis is probably antedated by the development of spherocytosis, although of this no complete evidence is available Hemolysis in a complex system is probably facilitated by other factors such as that of *p*H the amount of potassium or hydrogen ions the temperature and perhaps the vital activities of certain cells notably those in the spleen

Agglutinins Agglutinins are much more commonly found than hemolysins What is more the hemolytic activity of complex hemolysin is in part that of agglutinin (which is probably the same as amboceptor or sensitizing agent) Agglutinins when introduced into the circulation result in hemolysis of red cells They may be of different types some acting at or above body temperature (warm agglutinin e g the anti Rh agglutinin) some acting well at any temperature room icebox incubator (e g the iso agglutinins anti A and anti B) and some acting best at icebox temperature i e cold agglutinin Agglutinins may also be pure or simple in type without any but an agglutinative action or they may be more complex i e act as agglutinins in one set of circumstances but as hemolysins under other conditions e g when complement is added This is the case with colloidal silicic acid and immune agglutinin [7] A further differentiation of agglutinins has recently been made depending upon whether they act well when diluted with normal salt solution or whether their activity is masked in such a solution to be brought out only if whole blood plasma or albumin is used as a diluent An at

molysis, which is probably an active and not a passive process The exact mechanisms which are operative in a given case of hemolytic anemia are quite obscure, but attempts to uncover them should always be made

CLASSIFICATIONS OF HEMOLYTIC ANEMIA

I *Conventional or Nosographic Classification*

The classification which we have found most useful follows the conventional pattern

Hemolytic Syndromes

A *Hereditary*
1 Spherocytic (familial or congenital hemolytic jaundice or anemia)
2 Mediterranean target oval cell (including Cooley s anemia in mild forms)
3 Sickle cell (African target sickle cell)

B *Acquired*
1 Chemical origin—phenylhydrazine sulfonamides, etc
2 Bacterial origin—Streptococcus hemolyticus B coli etc
3 Parasitic origin—malaria, Oroya fever
4 Symptomatic origin—secondary or symptomatic of an underlying disease (Hodgkin s disease etc)
5 Idiopathic origin—with or without hemolysins or agglutinins hypersplenic types

C *Hemoglobinurias*
1 Paroxysmal cold a (Donath Landsteiner) hemolysin b Cold hemagglutinin
2 Paroxysmal march
3 Paroxysmal nocturnal (Marchiafava Micheli)
4 Others

II *Types of Hemolysis Reticulo endothelial* vs *Intravascular*

In general 2 types of hemolysis can be discriminated In one, there is a gradual disintegration of the red cell and its hemoglobin component This evidently takes place outside the circulating blood and may be brought about in various sinusoidal areas with and without the activity of reticulo endothelial cells This type of hemolysis is unaccompanied by an increase in the plasma hemoglobin concentration but an increase in plasma bilirubin takes place which is followed by an increase in the output of urobilinogen in the feces The spleen becomes enlarged at times excessively so evidently because its blood destructive function is greatly increased

In the second or intravascular type of hemolysis a mass of red cells is suddenly or violently disrupted within the circulating blood itself As a result there is a quick liberation of hemoglobin and a consequent increase in plasma hemoglobin If a sufficient amount of blood (roughly 30 cc or over) is thus hemolyzed the plasma hemoglobin concentration rises above the threshold level of 150 mg per 100 cc with the resultant passage of hemoglobinous urine Hemoglobinuria is thus indicative of violent intravascular hemolysis of more than 30 cc of blood Hemoglobinuria must always be accompanied by hemoglobinemia but the reverse is not

passive mechanism although it is possible that the passive factor may have some bearing What is more, under conditions of extreme erythrostasis we have found there is actually a decrease in hemolysis Furthermore the theory of erythrostasis has no bearing whatever in ca es with actual hemolysinemia as in paroxysmal noc turnal hemoglobinuria nor in March hemoglobinuria in which the reverse of stasis is present

Splenic activity The role of the spleen in normal and increased hemolysis is still obscure Although it is attractive to consider the spleen as the graveyard of the red blood cell this is by no means proved In almost all conditions with increased hemolysis the spleen is enlarged and in some of these splenectomy is followed by dramatic recovery Does the spleen become enlarged because it must remove more abnormal red cells from the circulation than normally, or does it enlarge as a primary dysfunction and thus result in hemolysis? It would appear that both answers are probably correct i e (1) the spleen often acts to remove red cells which have been partially hemolyzed elsewhere and (2) in certain hyper splenic cases the spleen appears to be primarily responsible for the increased he molysis and its removal results in complete cessation of the hemolytic state The hypersplenic cases are usually associated with leukopenia granulocytopenia and thrombocytopenia i e pancytopenia indicative according to our concepts of an unusual degree of inhibitory effect of the hyperactive spleen upon bone marrow for mation and delivery of the various cells Definite proof other than splenectomy for a hypersplenic type of hemolysis has thus far been lacking i e there is usu ally no histologic or other direct evidence obtained through extracts etc that the spleen is the initiator of the entire hemolytic picture Histologically certain cases show intense erythrophagocytosis It may be concluded that the spleen is certainly of aid in most hemolytic processes and that at times it initiates and carries through the entire reaction

Certain chemical and physical factors Certain chemicals notably saponin, lecithin arsine phenylhydrazine certain drugs containing the benzene ring and including the sulfonamide compounds acetanilid etc have the property of injuring the red cell and causing its hemolysis [11] Inorganic acids saturated fatty acids and their halogen derivatives certain alcohols also cause hemolysis These probably act on the red cells in different ways Physical factors including extreme heat, cold (in the presence of agglutinin) certain radiations including the ultraviolet (especially in the presence of eosin or other like dyes) etc may injure the red cell and result in its hemolysis [11] Contributing factors may be the pH the concentration of potassium sodium ugar etc in the solution [11]

Summary of pathogenetic mechanisms One may conclude that the red cell can be in jured in a variety of ways whether directly by a chemical factor or by hemolysis of simple variety or by heat or in a more complex fashion by an agglutination—hemolysin mechanism (immune hemolysin) or by the combination of agglutination and mechanical trauma These methods of hemolysis may be aided by such factors as erythrostasis an acid pH etc In any event the red cell is actively injured and either partial hemolysis (spherocytosis) or complete hemolysis results Erythrostasis the spleen and the pH are rarely the sole cause for the development of he

it is possible that the patient s own tissues produce specific substances which act only upon the patient s own red cells

Confirmatory of the presence of a substance causing spherocytosis of mature red cells is the extreme degree of this abnormality which develops during a hemolytic crisis The hypotonic fragility increases during this time, and there is leukopenia, neutropenia thrombocytopenia and reticulocytopenia These cytopenias suggest an active degree of hypersplenism Recent studies in our laboratory indicate that the destruction of normal red cells may become increased at this time In 2 cases an abnormal iso antibody was demonstrable during crisis

The rapid rate of hemolysis of introduced red cells in certain cases of acquired hemolytic anemia indicates a hemolytic constitution, i e a mechanism by which introduced normal red cells as well as those of the patient are destroyed at an abnormally rapid rate An exponential type of curve has been assumed by Brown et al [13] and has been confirmed in a few clinical observations [14] Our own studies indicate that the rapid hemolysis of normal red cells may proceed either exponentially or in a straight line fashion With the possible exception of the crisis, therefore the finding of a definitely decreased red cell life span indicates (1) acquired hemolytic anemia, and (2) a definite iso-antibody effect Such cases as a rule are associated with the presence in the serum of abnormal agglutinins or hemolysins which are usually of the immune body type (cf below)

Still a third type of hemolysis noncongenital definitely acquired but associated with a normal red cell life span was recently observed in a case of acute hemolytic anemia associated with chemical poisoning (refrigerant) In this case, no abnormal iso-antibodies of any type were present

IV *Types of Hemolysis as Determined from Study of Serum for Abnormal Iso-antibodies and from Study of Red Cells for Adsorbed Immune Antibody*

The cases of hereditary hemolytic disease (spherocytic target oval cell, target-sickle cell types) are not as a rule associated with the presence in the serum of demonstrable abnormal iso antibodies whether hemolysins or agglutinins An exception as noted above may be during the hemolytic crisis of familial spherocytosis

In cases of acquired hemolytic anemia except those due to chemicals and those which are symptomatic or secondary to some other condition such as Hodgkin s disease we have found an agglutinin which is apparently best brought out by the use of bovine albumin rather than normal salt solution as a serum diluent This agglutinin is usually of the cold variety and may be of high titre Its exact relationship to the hemolytic process is perhaps obscure since its reactions in vitro are best brought out at temperatures far below the normal body temperature The potentiation of hemolytic activity of cold hemagglutinin has been pointed out recently by Boorman et al [15] Some cases with a warm agglutinin may show a greatly increased activity in albumin solutions as compared with saline That this is due to the presence of a univalent blocking or incomplete antibody as claimed by Wiener[16] for anti Rh agglutinins is debatable We believe it more likely that the agglutinin activity is more readily brought out in solutions of albumin or in plasma

necessarily true since ordinarily plasma hemoglobin levels of less than 150 mg are not accompanied by hemoglobinous urine With the presence of an increased plasma hemoglobin level, an abnormal blood pigment called methemalbumin[12] is formed Small quantities of methemoglobin may also be produced The spleen may not become enlarged even with successive bouts of violent hemolysis apparently because red cell destruction is carried out chiefly within the circulating blood and not in the reticulo-endothelial sinusoids In paroxysmal nocturnal hemoglobinuria both types of hemolysis seem to take place simultaneously Intravascular hemolysis takes place with the patient in the supine position generally at night and normal or reticulo-endothelial hemolysis occurs during the rest of the day In this condition a mixed type of hemolysis is present with a resultant increase in both plasma hemoglobin and bilirubin levels, hemoglobinuria a slight increase in fecal urobilinogen and splenomegaly There is in addition a very unusual type of iron removal from the hemoglobin molecule with its deposition in renal tubules

III *Types of Hemolysis as Determined from Red Blood Cell Survival Studies*

Numerous studies of the life span of the red cell by various methods indicate that it has a longevity of approximately 110 to 140 days [1] In our laboratory we have used a modified Ashby technic utilizing high titre anti A anti B anti M anti N and anti Rh testing sera for differential agglutination directly in the red blood cell counting pipet By introducing normal red cells into the circulation of patients with hemolytic disease it is possible to discriminate at least 2 types of hemolysis In one the introduced red cells are destroyed in a normal linear fashion the life span being approximately normal This occurs in familial spherocytosis and in the other hereditary hemolytic syndromes of Mediterranean target oval cell disease and sickle cell disease In certain cases of acquired hemolytic anemia particularly in those associated with circulating iso-antibodies the curve of red cell destruction is a much faster one According to some investigators this curve is of the exponential variety In some of our cases however the curve has been linear in type although a rapid loss of red cells took place Thus in certain cases of hemolytic anemia the disorder is evidently one which centers largely around the patient s own abnormal red cells as a result of which the normal processes of hemolysis cause increased red cell destruction According to a number of investigators[13] this is the situation for example in familial spherocytosis in which the abnormal red cells are destroyed at a rapid rate by a normal spleen Normal red cells are destroyed at the normal slow rate When the spleen is removed increased red cell destruction ceases even though spherocytosis persists These observations apparently indicate that there is no abnormality in hemolysis per se but that the red cells themselves are abnormal This explanation does not elucidate the fundamental cause of the spherocytosis By analogy with our experiments concerning the production of spherocytosis and with the knowledge that the spherocyte represents a red cell which has been injured it would seem likely that the spherocyte of familial spherocytosis is a red cell which has been injured by hemolysin in the general sense Since normal red cells are not destroyed at a more rapid rate in the circulation of a case of this disease,

nuria [18] In this disease there is also an increased acid hemolysis One may tabulate these data as shown in table 1

TABLE 1 —*Differential Fragility Tests*

Types of Hemolytic Disease	Hypotonic Fragility	Mechanical Fragility	Acid Fragility	Heat Fragility
Hereditary				
Spherocytic	+	−	−	−
Target cell	+ (resistant)	−	−	−
Sickle cell	+ (resistant)	−	−	−
Acquired				
With agglutinins	+ or −	+	−	−
Without agglutinins	+ or −	−	−	−
Hemoglobinurias				
Paroxysmal cold (syphilitic)	−	−	−	+ (with cold)
Paroxysmal cold (with agglutinin)	+ or −	+	−	−
Paroxysmal nocturnal	−	−	+	+
Paroxysmal march	−	−	−	−

TABLE 2 —*Differentiation of Familial Spherocytosis from Acquired Spherocytosis*

Test used	Familial spherocytosis not in crisis	Familial spherocytosis in crisis	Acquired hemolytic anemia	Chemical
Red cell survival time	Normal	Decreased	Decreased ? exponential curve	Normal
Iso-antibodies	None	May be	Present	Absent
Anti human serum rabbit serum (Boorman Dodd Loutit)	Negative	?	+	Presumably negative
Mechanical fragility	Normal	?	+	Normal

A review in tabular form of some of the various tests which we have found useful in the differentiation of the familial sperocytic anemias from the acquired types is presented in table 2

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than in normal salt solution not because the agglutinins are incomplete, but because these solutions are more physiologic diluting materials than normal salt solution Erythroblastosis foetalis is an example of a congenital but *acquired* hemolytic disease due to the presence of abnormal agglutinins (usually of the anti Rh type) These may be complete, i e readily brought out in salt solution or incomplete, i e not brought out in salt solution but found only when albumin or plasma solutions are used for titration

Some cases of acquired hemolytic anemia show an iso-hemolysin which is of the immune body variety and is active against red cells of all blood groups as well as against the patient s own red cells [9]

Cases of acquired hemolytic anemia which are due to chemical poisoning or are secondary to such conditions as Hodgkin s disease lymphosarcoma etc do not show immune bodies of any type in the serum

Recently Boorman Dodd and Loutit[17] described an interesting test by which they discriminated between familial and acquired cases of hemolytic disease Beginning with the postulation previously advanced by Dameshek and Schwartz[9] that in certain cases of acquired hemolytic anemia the serum might be devoid of demonstrable iso-antibody which was however, adsorbed on the red cell, they prepared an anti human serum rabbit serum By using this serum againt red cells of acquired and hereditary cases they found positive results in the acquired cases and negative results in the hereditary types Negative results were also obtained in symptomatic and chemical cases Although these results are in accord with our previous postulations and with the results of red cell longevity studies with studies of serum iso-antibodies they require further confirmation before they can be completely accepted as to their reliability in differentiating between hereditary and acquired hemolytic disease

V *Types of Hemolysis as Determined from Differential Fragility Studies of the Red Cells*

The hypotonic fragility test is an index simply of the degree of thickness of the red cell The thicker the cell the greater is its fragility to hypotonic solutions of sodium chloride The red cell may be tested by other physical or chemical methods to determine possibly the type of substance which has reacted with it Thus by use of mechanical trauma with glass beads in a mechanical shaker (mechanical fragility) we can readily determine that red cells from one condition are hemolyzed at a far greater degree than normally [7] In our experience this indicates the previous activity upon the red cell of agglutinin or the sensitization phenomena (first step) of hemolysis by a complex hemolysin When a cold hemagglutinin is present the mechanical fragility test must be performed with cold solutions or the tubes surrounded by ice In certain hemoglobinurias a cold hemagglutinin is present and therefore an abnormal mechanical fragility is present In others there is a complex autohemolysin which requires cold for sensitization and warmth and complement for hemolysis (Donath Landsteiner hemolysin) In others heat alone causes hemolysis (heat fragility increased) this occurs in paroxysmal nocturnal hemoglobi

GENERALITIES ON THE NUCLEOLAR CONTENT OF SOME BLOOD CELLS

By I. GONZALEZ GUZMAN, M.D.

THE nucleolar apparatus of the blood cells has not received sustained and profound attention from morphologists and cytophysiologists, its structural significance and functional characteristics not being well known. For this reason I have devoted most of my scientific activity to the matter since 1923, trying to search into the problems of nucleolar physiology or physiopathology.

Nucleolar studies on blood cells require a thorough knowledge of the special technics which serve to show clearly the morphology of the nucleolar apparatus under different conditions of observation or experimentation. These procedures allow the study of volumetric, morphologic and structural characteristics of nucleoli, which is strictly necessary before attempting to generalize upon data obtained from the experimental cytology or the pathological morphology belonging to the different blood diseases.

Having in mind what has been mentioned, I shall presently show briefly the technics for nucleolar studies and the nucleolar characteristics that must be estimated and recorded to permit the proper interpretation of what is found.

I. TECHNICS FOR NUCLEOLAR STUDIES IN BLOOD CELLS

In 1923 I proposed a method permitting simultaneously differential counts and the determination of the number of white cells in the hemocytometer chamber as well as determination of the number and morphology of nucleoli contained in lymphocytes and monocytes or young cells belonging to other series. Description of the method follows:

Methyl Alcohol ⅓ Leishman or Giemsa Technic
(Gonzalez Guzman, 1923)

Diluting fluid	
Methyl alcohol (pure)	1 cc
Distilled water	2 cc
Leishman's stain	5–10 drops, or
Giemsa's stain	1–4 drops

With this fluid fill up white cell count pipets to dilute blood 1:20.
Observe in counting chamber. Preferably use water immersion objective and ocular 10× or 15×.

RESULTS

When the amount of stain is small, in the lymphocytes the cytoplasm appears a greyish blue, the nuclei blue and the nucleolar corpuscles very deep blue and all this permits a clear visibility. In the nuclei only the karyolymph is stained and its color is uniform. When Leishman's or Giemsa's amount is greater the cytoplasm is blue, the nuclei violet and the nucleoli very deep blue. The samples in which the

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nuclear stain is very deep must be rejected because this makes the perception of the nucleoli difficult With some practice and experience and having always the same staining solution one can employ the appropriate quantity in order to obtain the best degree of stain

In the monocytes the results are about the same but it is useful to point out that the nucleoli are very fine and that only the water immersion objective shows them clearly

The nuclei of the granulocytes are blue or violet according to the amount of stain used They do not show any chromatic structure their cytoplasm appears colorless or slightly stained blue and only the granulations are stained with some intensity and specificity the neutrophils are pinkish violet the eosinophiles wine red and the basophiles dull purple The description of pathological cells is not the object of this note

When the staining solution is of poor quality or the pipets are dirty or have been used for leukocytic counts employing acetic liquids granules and precipitates are produced which make the observation difficult Cleaning the pipets each third day first with ammonia then with distilled water and finally with pure methyl alcohol is recommended leaving them in the oven during the night so that they are dry the following morning

Thus it is possible to make the following in the counting chamber

1) White cell count (accurate)
2) Differential count (very accurate)
3) Nucleolar studies (nucleolar image nucleolar index accurate)

Years later I employed the Poggi Negri technic using as vital stain methylene blue Details of the technic follow

Supra Vital Methylene Blue Technic

(Gonzalez Guzman 1936 after Poggi Negri)

Diluting fluid

Isotonic saline solution	100 cc
Methylene blue	0 50 Gm

Dilute venous or capillary blood to [illegible] per cent

Mix

After 10–15 minutes centrifuge

The sediment is seen between slide and coverglass with water or oil immersion objectives

RESULTS

In the lymphocytes the cytoplasm is intensely blue and shows clearly a delicate spongy structure and sometimes some plasmosomes The nucleus is stained a pale uniform blue because only the karyolymph has been stained and the nucleoli appear deep blue contrasting very clearly with the pale background of the nucleus In the monocytes the nucleoli are somewhat difficult to see They show tones similar to those of the lymphocytes except that the cytoplasm is stained very faintly and shows a variable amount of granular inclusions stained violet or blue We will not discuss their relations with the apparatus of segregation which is stained a neutral red In the granulocytes the surface of the granulations or the

whole, is stained and also the nuclei with characteristics which we do not mention herewith In the myeloid leukemias it is advisable to shake the sediment gently in order to add some air and to observe it afterwards because of the easy decoloration produced by these bone marrow cells

Data obtained with this technic Nucleolar image (accurate), nucleolar richness (not accurate) nucleolar vacuoles in some instances

Finally, in the last 6 years I have been using Rio Hortega s double impregnation applied to the blood, blood forming organs and tissues which I summarize as follows

Double Argentic Impregnation

(For blood González Guzman 1940)

First stage Fixation and embedding

I Blood Gelatin Embedding
1 Put 0 5 cc of capillary or venous blood into the following fluid
Isotonic saline solution 4 5 cc
Formaldehyde 40 per cent 0 5 cc
2 Allow 24–48 hours for fixation Shake once or twice a day
3 Centrifuge wash the sediment once with ammonium hydroxide 3 per cent and then with distilled water Centrifuge
4 Mix at 50 C one part of sediment with two of gelatin Difco 30 per cent 15 minutes
5 Pour into porcelain cuvett to clot Refrigerate at 1 C Evaporate on blotting paper at room temperature 30 minutes
6 Keep in formalin 5 per cent
II Blood-Clots or Tissues
Fix 3–5 mm thick sections in formalin 10 per cent for 24–72 hours

Second stage Coloration

I Frozen sections 10–15 micra thick (Blood embedding clots or tissues)
II Wash with ammonia water 3 per cent
III Wash in distilled water
IV Immerse in silver nitrate 2 per cent 24 hours at room temperature and under light
V Wash in the following fluid
Distilled water 50 cc
Pyridine 5 drops
VI Immerse in the following solution
Rio Hortega s silver carbonate 10 cc
Pyridine 2 to 6 drops
24 hours at room temperature and under light
VII Wash 10–20 seconds in distilled water
VIII Alcohol Creosote
IX Mount in Canada balsam

NOTES AND RESULTS

When blood inclusions the clots or the tissues once fixed are kept in formaldehyde for a long time their possibilities for nucleolar stain diminish It is better to stain them before a month Commercial silver nitrates give variable results according to the mark and even to the series of production It is advisable to use several of them and select the best The nucleolar stains are more beautiful if silver carbonate is used and prepared the same day Those which have been on hand more than

a week must not be used Pyridine added to the silver carbonate prevents staining of the chromatin and helps staining of the nucleoli If used in small doses chromatinic structures appear if used in greater doses only the cytoplasm is stained and in the nuclei the borders are not very clear For the same substances the most suitable doses of pyridine must be found or preferably stain 2 or 3 series of sections using variable doses of pyridine

In the best slides the cytoplasm has a variable intensity of yellow or light sepia In the light sepia nuclei and in the nucleoli the fundamental substance is likewise a darker sepia and the argentophylic grains are black The borders and the contrasts are not very clear in the palely stained sections In the overstained ones the chromatin and the intensity of the nucleolar stain make the perception of their internal structure difficult

Data obtained with this technic Nucleolar image very accurate Rn index very accurate structure of nucleolar bodies very clear

The first of the technics mentioned allows a general study of the leukocytes as well as the count of their nucleolar corpuscles The second one permits a finer cytological study of the leukocytes and the observation of nucleolar vacuolae The double argentic impregnation is the only method that facilitates the study of the internal structures of the nucleoli as well as the volumetric measurements and finest details of the general morphology

2 NUCLEOLAR CONSTANTS OF THE BLOOD CELLS

Nucleolar attributes may, therefore be measured and expressed numerically to constitute a characterization of the blood cells This characterization refers mainly to the number of nucleoli within a cell to the relation between nucleolar and nuclear volume and to the internal structure of the nucleolar bodies themselves

The nucleolar number has been particularly studied in the circulating lymphatic cells and the figures obtained have been expressed as the nucleolar image Nucleolar image is then only a classification of the lymphatic cells according to their nucleolar content and the determination of the number of nucleoli in 100 cells

Volumetric data pertaining to the nucleoli and nucleus can be made to constitute a ratio similar to that between nucleus and cytoplasm the ratio has been designated as Rn index since it expresses the nucleolar richness of cells

Finally the internal structure of the nucleoli gives important data since structure is modified in different pathological conditions The nucleolar constants are expressed as follows

Nucleolar Constants in a Given Type of Cell

1 The nucleolar image Grouping of cells in classes according to the number of nucleoli in the cell

2 Nucleolar index Number of nucleolar corpuscles seen in 100 cells

3 Nucleolar richness Expressed by Rn (nucleolar richness Riqueza nucleolar) is the volumetric relationship bet een the nucleolar mass and the olume of the nucleus To express it in figures near unity the following index is calculated

$$\text{Rn Index} \; \frac{\text{Nucleolar volume} \times 100}{\text{Nuclear volume}}$$

4 Number of argentophilic granules contained in the nucleolar bodies

In normal conditions all this data changes within narrow limits

In circulating blood cells and in those of the hemopoietic organs, nucleolar constants are related to cellular youth A cell has more nucleoli if it is young and the younger cells have a greater nucleolar content At the same time nucleolar structures and cellular youth are closely related A higher number of nucleolar bodies corresponds to a shift to the right in the nucleolar image and a greater nucleolar index, a larger volume of nucleoli corresponds to an increase of the Rn index or nucleolar richness, and nucleolar structures change due to an increase of the fundamental substance of the corpuscle and a greater number of the argentophilic grains

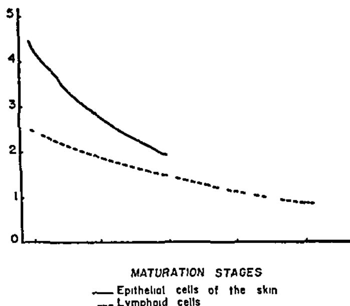


FIG 1 NUCLEOLAR RICHNESS EXPRESSED BY Rn INDEX

The nucleolar image and its condensed expression the nucleolar index is very useful *to evaluate the cellular youth of a given series but may not* be *considered* as accurate when it is to be compared with different classes of cells Nucleolar richness as expressed by the Rn index is perhaps the most reliable index of youthfulness *of a given type of cell and is also* very valuable when it is intended to compare different types of cells

In those cellular elements of limited life span which allow the identification of a juvenile stage another of maturity and a last one of senility it is possible to determine the Rn index during the different life stages and translate the figures to a co ordinate system writing as ordinates Rn values and in the abscissa line the successive stages of the cellular lives

3 DATA OF NUCLEOLAR RICHNESS AND CELLULAR YOUTH

When quantitative data on nucleolar richness in a given type of cell is made through its different stages of maturity and the Rn values are written as ordinates

and the evolutive stages as abscissas a curve is obtained, corresponding to an exponential function

The trend is frequently a hyperbola asymptotic and in most cases equilateral In figure 1 the trend in the cases of epithelial cells of the skin and lymphoid cells of the appendix is illustrated

4 SOME DATA ON THE LYMPHOID SERIES

The facts already mentioned have been amply studied and confirmed for the different blood cells From them I shall presently show the findings in 2 different types of blood cells the lymphoid and the erythroblastic

The nucleolar lymphocytic image has been determined first in normal adults and in relation to different physiological and pathological conditions In the following table I have condensed my findings and those of my associates

The Nucleolar Lymphocytic Image in Normal Conditions

	Classes					Nucleolar Index	Authors
	0	1	2	3	4		
Normal adults	1	92–96	3–6	1	0 0	104–110	Gonzalez Guzman
Infants	0 2	81 0	14 8	3 0	1 0	123 6	Arias Jose
Old age	2 5	94 0	3 5	0 0	0 0	101 0	Gonzalez Guzman
Pregnancy	0 2	87 0	11 4	1 2	0 0	113 4	Arias Juan
Puerperium	0 3	92 0	7 2	0 5	0 0	107 9	Roa
Digestive state	0 2	93 8	5 6	0 4	0 0	106 2	Vara

In a like manner I have condensed data in relation to the findings in different pathological conditions

The Nucleolar Lymphocytic Image in Some Pathological Conditions

	Classes					Nucleolar Index	Authors
	0	1	2	3	4		
Intestinal worms	0 4	89 0	10 6	0 0	0 0	110 2	Velazco
Cancer	0 5	91 0	8 2	0 3	0 0	108 3	Guevara
Leprosy	1 8	88 2	9 0	0 5	0 5	109 7	Mart Barragan
Tuberculosis	0 3	66 2	23 3	6 0	3 7	145 6	Pernas
Brucellosis	0 5	89 8	9 0	0 7	0 0	110 0	Gonzalez Guzman
Lymphatic leukemias							
Acute	0 0	23 0	40 0	28 0	9 0	223 0	Gonzalez Guzman
Sub acute	0 0	[illegible] 0	13 0	9 0	6 0	149 0	Gonzalez Guzman
Chronic	0 0	87 0	[illegible] 0	4 0	2 0	121 0	Gonzalez Guzman

Rn Index as expression of the nucleolar richness and the most valuable index of cellular youth has also been determined in normal conditions and in numerous patients Data so far obtained is as follows

Nucleolar Richness Rn Index In Normal and Pathological Conditions
(Gonzalez Guzmán)

	M	S d σ ±
Normal adults	0 96	0 22
Leprosy		
Tuberculoid	1 03	0 24
Lepromatous	1 06	0 25
Lucio s form	1 03	0 26
Mixed forms	1 04	0 26
Average	1 05	0 25
Brucellosis	1 23	0 27
Lymphoid leukemias		
Chronic	1 15	0 30
Sub acute	1 40	0 33
Acute	1 90	0 40

Also studies have been made on lymphocytic infiltrates of tumoral stromas, determining their nucleolar richness The following table shows my findings

Nucleolar Richness Rn Index in the Lymphocytes of the Tumoral Stromas

Type of cancer	Rn Index	
	M	σ ±
Carcinoma of the skin		
Basal	0 62	0 25
Squamous	0 60	0 25
Carcinoma of the uterus		
Cervix	0 64	0 26
Body	0 65	0 24

Previous data on the lymphoid series, allows the following conclusions

1 From the nucleolar standpoint there may be added to the known lymphocytic constants the 3 following ones the lymphocytic nucleolar image the Rn index and the number of argentophilic grains of the nucleolar corpuscles

2 In normal conditions circulating lymphocytes of infants and children are younger than those of adults The opposite is true in the case of old age

3 Pregnancy puerperium and the different moments of the digestive process do not significantly alter the nucleolar lymphocytic image

4 In pathological conditions there is observed an increase of the nucleolar index in tuberculosis and particularly in lymphatic leukemias In the latter the increase is related to the rapidity of evolution of the disease and the severity of the prognosis

5 Nucleolar richness of the lymphoid cells in cases so far studied is increased in brucellosis and in lymphatic leukemias and diminished in the lymphocytic infiltrates in tumoral stromas Data gathered in cases of leukemias have a definite prognostic value

The findings mentioned may prove of value in helping in the clinical diagnosis or prognosis of some diseases

5 SOME DATA OF THE HEMOGLOBINIC SERIES

Investigations parallel to those described about the lymphoid cells have been made on the nucleated cells of the hemoglobinic series of mammals and on nucleated red cells of birds and low vertebrates, and particularly on amphibian and reptiles Studies of the more immature forms of embryonic blood have also served as basis for the investigations pursued

Important facts from these investigations are the following

1 That the nucleolar erythroblastic image shows a shift to the right classes 2 3 4 in the younger forms of this series The shift is to the left Classes 0 1 in the more mature cells with hemoglobinic cytoplasm

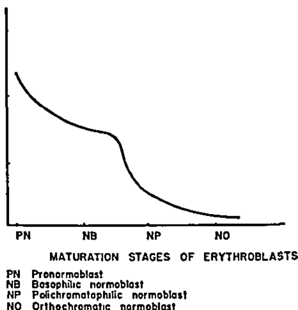


FIG 2 NUCLEOLAR RICHNESS EXPRESSED BY Rn INDEX

2 Nucleolar richness expressed by the Rn index shows a progressive descent as the cell ripens and its cytoplasm charges with hemoglobin

3 In the very young forms such as promegaloblasts basophilic megaloblasts pronormoblasts or basophilic normoblasts the nucleoli have a relatively complex structure with abundant funadamental substance and many argentophilic grains while in the more mature forms with polychromatic reddish or orthochromatic cytoplasm the fundamental substance has disappeared and only one or two argentophilic grains are seen

4 A very important observed fact points out that when the pronormoblasts or premegaloblasts start their cytoplasmic ripening process that leads to the formation of hemoglobin the nucleolar corpuscles lose their rounded or ellipsoidal form and take instead an irregular shape giving the impression that the colloids of their

fundamental substance have been transformed into a more fluid substance that tends to mix with the karyolymph This assertion finds support in the fact that in later evolutive phases the fundamental substance disappears and there persist only some free or encrusted argentophilic grains

5 The nucleolar curves during the maturing process are extremely important In mammals they start with the known exponential trend, but a little after the starting point a sudden descent of the curve shows up, as if another exponential function was superimposed on the first one, leading the tracing to zero

In amphibians the phenomenon is essentially the same, there is the imbrication of two tracings, but the second one does not reach the zero point, descending in stead to constitute a small segment of the curve parallel to the axis of the abscissa and very close to it In other words, in amphibians nucleoli do not disappear in mature red blood cells, remains of them persisting as fine granular corpuscles visible only by employing some of the technics already described

Birds occupy an intermediate position, some mature red cells have already lost their nucleolar material while the majority still show a very fine dusty corpuscle

The facts mentioned are expressed graphically in figure 2 and permit us to state the following conclusions and working hypothesis

1 During the maturative process of the hemoglobinic series there exists a decrease of the nucleolar richness whose graphic expression in common to the other type of cells corresponds to an exponential function

2 Shortly after the first trend is constituted there appears a sudden change in the slope that corresponding to another exponential trend leading the curve to zero or very near to the axis of the abscissas

3 The new tracing corresponds to the time where the formation of hemoglobin starts being so considered as the graphic expression of the starting of this phenomenon

4 The evolution of the nucleolar richness and of the morphology of the nucleolar corpuscles is suggestive of the following hypothesis that the fundamental substance of the nucleoli suffers a series of physical and chemical changes that transform it into another substance forerunner of hemoglobin that diffuses first into the karyolymph passing later to the cytoplasm to be changed into hemoglobin Two complementary questions arise immediately Is the transformation of the nucleolar substance a self made process starting in the nucleolar substance and ending in hemoglobin? Or is the forerunner substance of hemoglobin of nucleolar origin transformed into hemoglobin by the activity of the mitochondrial apparatus? The latter asumption seems more likely in the light of studies now under progress in erythroblasts of chicken embryos

The findings mentioned may prove of value in helping in the clinical diagnosis or prognosis of some diseases

5 SOME DATA OF THE HEMOGLOBINIC SERIES

Investigations parallel to those described about the lymphoid cells have been made on the nucleated cells of the hemoglobinic series of mammals and on nucleated red cells of birds and low vertebrates and particularly on amphibian and reptiles Studies of the more immature forms of embryonic blood have also served as basis for the investigations pursued

Important facts from these investigations are the following

1 That the nucleolar erythroblastic image shows a shift to the right classes 2, 3 4, in the younger forms of this series The shift is to the left Classes 0 1 in the more mature cells with hemoglobinic cytoplasm

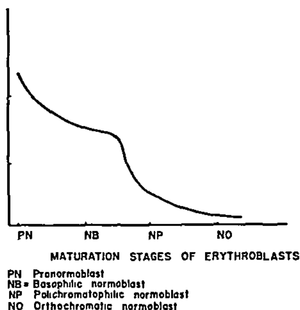


FIG 2 NUCLEOLAR RICHNESS EXPRESSED BY Rn INDEX

2. Nucleolar richness expressed by the Rn index shows a progressive descent as *the cell ripens and its cytoplasm charges with hemoglobin*

3 In the very young forms such as promegaloblasts basophilic megaloblasts pronormoblasts or basophilic normoblasts the nucleoli have a relatively complex structure with abundant funadamental substance and many argentophilic grains while in the more mature forms with polychromatic reddish or orthochromatic cytoplasm the fundamental substance has disappeared and only one or two argentophilic grains are seen

4 A very important observed fact points out that when the pronormoblasts or premegaloblasts *start their cytoplasmic ripening process that leads to the formation of hemoglobin* the nucleolar corpuscles lose their rounded or ellipsoidal form and take instead an irregular shape giving the impression that the colloids of their

tinins have to be neutralized with A and B substances in order to demonstrate anti Rh antibodies or any other abnormal antibodies which might be present in the serum under investigation We know now that besides the anti Rh agglutinin a different type of Rh antibody the incomplete or blocking antibody (Race Wiener) might be present By using as a diluent undiluted serum or albumin solution instead of saline solution (Diamond and Abelson Wiener), this blocking antibody too will agglutinate Rh positive cells It is advisable to use both saline solution as well as some other diluent such as albumin solution or undiluted serum in testing the patient s serum against the husband s cells in a case of suspected erythroblastosis Table 1 illustrates the experimental setup used by us under those circumstances

TABLE 1 — *Agglutination of Blood Cells Group A (Mr Ba) by Non neutralized and Neutralized Serum of Group O (Mrs Ba)*

Serum Mrs Ba	Part I Non neutralized		Part II Neutralized	
	a	b	a	b
	Dilutions in			
	Saline	Serum	Saline	Serum
1 3	++++	++++	—	—
1 6	++++	++++	—	—
1 12	++++	++++	—	—
1 24	++++	+++	—	—
1 48	+++	+++	—	—
1 96	++	++	—	—
1 192	+	+	—	—
1 384	±	±	—	—
1 768	—	—	—	—
0	—	—	—	—

— = no agglutination ± = faint agglutination + = slight agglutination ++ = marked agglutination +++ = strong agglutination ++++ = very strong agglutination

Mrs Ba is Rh negative group O Mr Ba is Rh positive group A and her serum is to be examined for Rh antibodies The experiment given in table 1 consists of 2 parts In the first part Mrs Ba s serum is not neutralized In the second part Mrs Ba s serum is neutralized by the addition of 2 parts of AB substances to 1 part of her serum In rows (a) serum dilutions and blood cell suspensions were made in saline solution In rows (b) serum dilutions and blood cell suspensions were made in undiluted serum of Group AB The tubes were kept for one hour at room temperature and then centrifuged at medium speed The resulting agglutination is shown in table 1

Table 1 shows that there is no difference in the anti A titer of Mrs Ba s serum irrespective of whether the dilutions are made in saline solution or in serum Following neutralization with AB substances the anti A antibody is completely eliminated Therefore there is no Rh antibody or any other abnormal antibody detectable in Mrs Ba s serum against Mr Ba s cells

INTERRELATIONSHIP BETWEEN THE Rh SYSTEM AND THE A B SYSTEM

By Ernest Witebsky M D

(*With the technical assistance of* Mrs Livia Blum Miss Doris Howles and Miss Helen Ward)

I

IT IS generally understood that the Rh system and the AB system are entirely independent of each other In spite of their complete independence however only the full understanding of both the AB system and the Rh system make it possible to interpret and to correlate clinical manifestations and test tube observations A sensitized Rh negative patient might suffer a fatal hemolytic transfusion reaction just as well if blood of the incorrect blood group is transfused as if Rh positive homologous blood is given Barely 7 years have elapsed since the Rh factor was discovered and we are certainly far from having reached a complete understanding of this highly complicated and involved subject It is now 47 years since Landsteiner first discovered the major blood groups and yet those who believe that we know everything about them are badly mistaken

As an example indicating the close interrelationship existing between the Rh system on the one hand and the AB system on the other I would like to point to the production of Rh test serum Rh test sera used at the present time are prepared from human blood obtained from Rh negative patients sensitized to the Rh factor either by transfusion of Rh positive blood or by a fetus whose blood cells entered the mother s circulation stimulating antibody production in that way Human serum of course contains the isoagglutinins anti A and anti B depending upon the blood group to which the respective individual belongs The elimination or neutralization of those isoantibodies is a prerequisite in the production of reliable anti Rh sera Unless completely eliminated these isoagglutinins will obscure the diagnosis of the Rh factor and give rise to false positive reactions The isoagglutinins can be removed either by absorption with Rh negative A cells and Rh negative B cells or by neutralization with the isolated blood group specific substances A and B * This technic has been used in the past by us as well as by others and has been found to be satisfactory

In cases of suspected erythroblastosis we have been in the habit for many years of testing the wife s serum against her husband s cells whenever feasible If the wife s serum contains isoagglutinins against her husband s cells these isoagglu

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Purified blood group specific substances A and B as used in this paper have been kindly supplied first by the Eli Lilly Company and later by Sharp & Dohme In most of the experiments 10 cc vials were used containing solutions of A and B specific substances in dilution of roughly 1 2000 They consist of mixtures of A substances isolated from the gastric mucosa of hogs and AB substances isolated from the gastric mucosa of horses

speed The resulting agglutination is given in table 3 According to this experiment, Mrs Bong s anti A titer is 256, her anti B titer 128 In our hands these titers are considered to be average or slightly above average

In order to test Mrs Bong s serum for Rh antibodies, using Mr Bong s cells the isoagglutinin anti A in Mrs Bong s serum had to be neutralized by the addition of AB substances Table 4 shows an experiment which was carried out in order to determine the minimum amount of AB substances necessary to neutralize completely the isoagglutinins anti A and anti B of Mrs Bong s serum Three mixtures were prepared The first consisted of 2 parts serum Bong + 1 part AB

TABLE 3 —*Determination of Anti A and Anti B Titer of Serum Mrs Bong*

Serum Mrs Bong Rh Negative Group O	a A Cells	b B Cells
1 4	++++	++++
1 8	++++	++++
1 16	++++	+++
1 32	+++	+++
1 64	++	++
1 128	+	+
1 256	+	−
1 512	−	−
0	−	−

TABLE 4 —*Determination of Minimum Amount of AB Substances Necessary to Neutralize Serum Mrs Bong*

	Mixtures to be tested					
	Mixture One		Mixture Two		Mixture Three	
	a A Cells	b B Cells	a A Cells	b B Cells	a A Cells	b B Cells
Undiluted	+	+	−	−	−	−
1 2	+	±	−	−	−	−
1 4	±	−	−	−	−	−
1 8	−	−	−	−	−	−
0	−	−	−	−	−	−

substance the second consisted of 1 part serum Bong + 1 part AB Substance the third consisted of 1 part serum Bong + 2 parts AB Substance

The mixtures were kept at room temperature for 30 minutes To decreasing dilutions of the mixtures (volume 0 1 cc) were added then in rows (a) 0 1 cc test group A cells 2 per cent suspension in rows (b) 0 1 cc of test group B cells 2 per cent suspension The tubes were kept at room temperature for 1 hour and then centrifuged at medium speed The resulting agglutination is given in table 4

Table 4 shows that the mixture of equal parts of serum Mrs Bong and AB substances results in the complete neutralization of the isoagglutinins anti A and anti B After having determined the minimal amount of AB substances necessary

The second table is given only as an example to illustrate the agglutination of Rh positive cells by the Rh blocking antibody in the presence of a suitable diluent such as undiluted human serum as it is practiced in our laboratory at the present time

Decreasing amounts of serum of Mrs Poll Rh negative group O (volume 0 2 cc) were mixed with 0 2 cc of Rh positive group O cells subtype Rh_1 (2 per cent suspension) In row (a) all dilutions including the cell suspension were made in saline solution In row (b) all dilutions, including the cell suspension were prepared in undiluted human serum The tubes were kept for 60 minutes in the water bath at 37° C and centrifuged at medium speed for one minute The resulting agglutination is recorded in table 2 This experiment shows the presence of a

TABLE 2 —*Influence of the Diluent upon the Agglutination of Rh Positive Cells by Anti Rh Serum Containing Rh Blocking Antibodies*

Serum Poll Group O Rh Negative	a All dilutions in saline	b All dilutions in serum
Undiluted	+	++
1 2	−	++
1 4	−	++
1 8	−	+
1 16	−	+
1 32	−	+
1 64	−	+
0	−	−

blocking antibody of medium potency which manifests itself only if undiluted human serum is used as a diluent instead of physiological saline solution

II

The observations to be reported in this presentation have been stimulated by a a patient whose history is as follows

Mrs Bong 29 year old housewife had 3 previous pregnancies 1941 normal male infant 1942, premature labor at 6 months duration of pregnancy female infant weighing 1 lb 2 oz said to have lived 10 minutes 1943 normal 4 hour labor with dead born female weighing 9 lb 10 oz post mortem findings typical of erythroblastosis Mr and Mrs Bong were sent to us by the attending obstetricians for blood examination regarding the Rh situation *

Our findings were as follows Mrs Bong blood group O Rh negative Mr Bong blood group A Rh positive subtype Rh_1 Table 3 shows the isoagglutinin titer of Mrs Bong s serum when titered against test cells of group A and of group B The experiment was carried out in the following way Decreasing dilutions of Mrs Bong s serum (volume 0 1 cc) were mixed with 0 1 cc each of (a) group A cells 2 per cent suspension (b) group B cells 2 per cent suspension The tubes were kept at room temperature for 20 minutes and then centrifuged at medium

* We are very much indebted to Dr James King and to Dr Milton Kahn for their kind co-operation

cells and absent in Mrs Bong s who belongs to the blood group O In order to test the second possibility, the following experiment was set up

Decreasing dilutions of Mrs Bong s serum neutralized by the addition of 2 parts of AB substance to 1 part of serum as in Experiment 5, were mixed with 2 per cent suspensions of (a) Mr Bong s blood cells group A, Rh_1, (b) test cells group A Rh_1, (c) test cells group A Rh negative (d) test cells group O, Rh_1 Serum dilutions as well as blood cell suspensions were made in normal serum of group A The tubes were kept for 60 minutes in the water bath at 37°C, centrifuged at medium speed for 1 minute and read for agglutination (table 6) This experiment shows that A cells are agglutinated by Mrs Bong s neutralized serum irrespective of the Rh factor Rh negative A cells as well as Rh positive A cells being agglutinated Rh_1 cells belonging to group O however, are not agglutinated The agglutination of A cells by Mrs Bong s serum under the circumstances described and the lack of agglutination of blood cells which did not contain the A factor was confirmed in a

TABLE 6 —*Agglutination of Human Cells by Neutralized Serum of Mrs Bong*

Serum Mrs Bong Neutralized	Blood Cells			
	Mr B ng Rh Group A	Rh_1 Group A	Rh− Group A	Rh_1 Group O
Undiluted	+++	+++	++	−
1 2	++	++	++	−
1 4	++	++	++	−
1 8	++	++	+	−
1 16	+	+	++	−
1 32	+	+	+	−
1 64	+	±	+	−
0	−	−	−	−

larger series of investigations Obviously therefore Mrs Bong s serum contains an anti A antibody of a new type which in some respects compares with the Rh blocking antibody being demonstrable only if physiological saline solution is replaced by normal serum as the diluent The peculiar nature of the anti A antibody present in Mrs Bong s serum suggested the possibility that this antibody like an immune antibody might possibly fix complement The isoagglutinins anti A and anti B occurring in normal human serum are characterized by their failure to give precipitation or complement fixation when mixed with water soluble A and B specific substances Some reports in the older literature claiming observations to the contrary could never be confirmed in our hands As antigens for the complement fixation test amniotic fluids whose content in A or B specific substances had been previously determined by their ability to neutralize the isoagglutinins anti A or anti B respectivel were selected The experiment itself was carried out in the following way

Decreasing dilutions of (a) amniotic fluid of group A (b) amniotic fluid of group B (volume 0 1 cc) were mixed with 0 1 cc of 1 20 diluted guinea pig serum To Part I 0 1 cc of 1 5 diluted inactivated serum Mrs Bong was added and to Part

to neutralize completely the isoagglutinins of Mrs Bong s serum we decided to add 2 parts of AB substances to 1 part of her serum about twice the amount found necessary Mrs Bong s serum thus neutralized could be examined for the presence of Rh antibodies or any other abnormal antibodies which might act upon antigens present in Mr Bong s blood cells The experiment itself was carried out in the following manner

Decreasing dilutions of Mrs Bong s neutralized serum (volume 0 2 cc) were mixed in Part I with 0 2 cc of Mr Bong s blood cells (2 per cent suspension) in Part II with 0 2 cc of test cells of group O, subtype Rh_1 (2 per cent suspension) *All* dilutions were prepared in rows (a) with physiological saline solution and in rows (b) with undiluted normal serum of group A The tubes were kept for 60 minutes in the water bath at 37°C and then centrifuged at medium speed for 1 minute The resulting agglutination is shown in table 5

TABLE 5 —*Agglutination of Mr Bong s Blood Cells (Group A Rh_1) by Mrs Bong s Neutralized Serum (Group O Rh Negative)*

Serum Mrs Bong Rh Negative Group O Neutralized	Part I Cells Mr Bong (Group A Rh)		Part II Test Cells (Group O Rh)	
	a	b	a	b
1 3	−	+++	−	−
1 6	−	+++	−	−
1 12	−	+++	−	−
1 24	−	+++	−	−
1 48	−	+	−	−
1 96	−	+	−	−
1 192	−	+	−	−
0	−	−	−	−

The experiment shows the following (1) In row (a) of Part I in which Mrs Bong s serum was matched against Mr Bong s cells no agglutination whatsoever occurred indicating the fact that Mrs Bong s serum was completely neutralized The diluent used in this row was physiological saline solution (2) Mrs Bong s serum though completely neutralized agglutinated Mr Bong s cells up to a dilution of 1 192 provided all dilutions are made in normal serum of group A (row (b) Part I) (3) The agglutination of Mr Bong s cells by Mrs Bong s neutralized serum would be perfectly understandable if one were to assume the presence of an Rh antibody in Mrs Bong s serum This assumption is not substantiated by the second part of the experiment in which Rh positive cells of the same subtype namely Rh_1 group O were used These cells failed to become agglutinated even under *conditions in which a blocking antibody should produce agglutination* (row (b) Part II)

Two possibilities had to be considered the first one being that there was a difference between Mr Bong s Rh type and that of the test cells used in the experiment and the second that the antibody present in Mrs Bong s serum was not an Rh antibody at all but was an antibody directed against the A factor present in Mr Bong s

phenomenon at all, or only to a very small extent, indicating the fact that the particular antibody occurring in Mrs Bong's serum is directed against the A_1 property rather than the A property as present in A_2 cells Furthermore, group B cells are not agglutinated either

The nature of the anti A antibody present in Mrs Bong's serum suggested the possibility of isoimmunization In that case one had to assume that Mrs Bong was sensitized toward the A factor during pregnancy and that her children had inherited the A factor from their father We therefore included in our investigations sera of patients who had received A and B substances either by the injection of pooled plasma or by the transfusion of O blood containing the purified A and B substances The sera of many patients of that type were at our disposal As an example the serum of patient Wall, who received 1000 cc of pooled plasma, as well

TABLE 8 —*Agglutination of Human Blood Cells by Non neutralized and Neutralized Serum of Mrs Bong*

Part I Serum Bong non neutralized diluted in saline solution
Part II Serum Bong neutralized diluted in saline solution
Part III Serum Bong neutralized diluted in normal human serum Group A

Serum Mrs Bong	Blood Cells								
	Part I			Part II			Part III		
	a A	b A	c B	a A	b A_2	c B	a A_1	b A_2	c B
1 5	++++	++++	++++	+	−	−	++++	+	±
1 10	++++	++++	+++	+	−	−	+++	+	−
1 20	++++	++++	+++	±	−	−	+++	+	−
1 40	+++	+++	++	−	−	−	+++	−	−
1 80	+++	++	+	−	−	−	+++	−	−
1 160	++	++	±	−	−	−	++	−	−
1 320	++	+	−	−	−	−	++	−	−
1 640	+	−	−	−	−	−	+	−	−
1 1280	−	−	−	−	−	−	−	−	−
0	−	−	−	−	−	−	−	−	−

as 1000 cc of group O blood conditioned with A and B specific substances was selected The experiment was carried out in the following way

Decreasing dilutions of serum Wall who belonged to the blood group O (volume 0 2 cc) were mixed with 0 2 cc of 2 per cent suspension of A_1 cells and B cells respectively In Part I of the experiment native serum Wall was used in Part II serum Wall was neutralized by the addition of 2 parts of AB substances to 1 part of the serum The experiment was carried out in duplicate namely in physiological saline solution as well as in normal human serum of group AB The tubes were kept at room temperature for 1 hour and then centrifuged at medium speed for 1 minute The resulting agglutination is shown in table 9 This experiment reveals the following facts (1) Serum Wall agglutinates A_1 and B cells fairly strong in physiological saline solution the anti A titer being 1536 the anti B titer 768 (2) The anti A_1 titer of serum Wall is increased by using human serum as a diluent instead of physiological saline solution However the anti B titer is not substan

II 0 1 cc of 1 5 diluted inactivated normal serum group O After standing for 1 hour in the icebox and an additional hour in the incubator at 37°C , 0 2 cc of sensitized sheep cells were added The resulting hemolysis is shown in table 7

The serum of Mrs Bong fixes complement in the presence of A substance, but not of B substance in spite of the fact that her serum contains both isoantibodies anti A and anti B In contrast the normal or natural isoantibodies occurring in human serum group O do not fix complement suggesting an additional difference between the normal isoantibody anti A and the anti A antibody present in Mrs Bong s serum

TABLE 7 —*Complement Fixation Test*
Part I *Serum Mrs Bong Group O*
Part II *Normal serum Group O*

Amniotic Fluid	Part I		Part II	
	a Group A	b G o p B	a G o p A	b G oup B
Undiluted	++++	−	−	−
1 3	++++	−	−	−
1 9	++++	−	−	−
1 27	+++	−	−	−
1 81	±	−	−	−
1 243	−	−	−	−
0	−	−	−	−

− complete hemolysis ± almost complete hemolysis +++ trace of hemolysis ++++ no hemolysis

III

The question arose whether the peculiar anti A antibody as demonstrated in Mrs Bong s serum was acting upon the A factor as such or whether there was a difference regarding the subgroups of A For this reason the following experiment was carried out

Decreasing dilutions of serum of Mrs Bong (volume 0 2 cc) were mixed with (a) 0 2 cc of 2 per cent blood cell suspension group A_1 (b) 0 2 cc of 2 per cent group A_2 suspension (c) 0 2 cc of 2 per cent group B suspension The experiment consisted of three parts In Part I native serum Mrs Bong was used meaning that it was not neutralized and all dilutions and cell suspensions were made in physiological saline solution In Part II serum Mrs Bong was neutralized by adding 2 parts of AB substances to 1 part of serum and all dilutions again were made in physiological saline solution In Part III serum of Mrs Bong was neutralized as in Part II *except that normal human serum of group A was used as a diluent for* all dilutions and blood cell suspensions The tubes were kept for 1 hour at room temperature and then centrifuged at medium speed for 1 minute The resulting agglutination is shown in table 8 According to table 8 A_1 cells are agglutinated by the neutralized serum of Mrs Bong provided all dilutions are made in undiluted normal human serum (Part II row (a)) In contrast A cells do not show the

AB substances to 1 part of serum In rows (a) and (b) all dilutions were made in physiological saline solution In rows (c) and (d) all dilutions were made in *undiluted human serum of group A* The tubes were kept for 60 minutes at room temperature, centrifuged and read for agglutination (table 10)

Both A_1 and A cells are agglutinated up to a dilution of 1 3072 by serum Uge diluted in saline solution (Part I a and b) The agglutination of A cells is somewhat weaker than that of the A_1 cells However this difference is considerably increased if normal human serum is used as a diluent instead of saline solution As

TABLE 10 —*Agglutination of A_1 and A_2 Cells by Anti A Immune Antibody*

Serum Uge Group B	Blood Cells							
	Part I Not Neutralized				Part II Neutralized			
	a A	b A_2	c A	d A_2	a A_1	b A_2	c A	d A_2
	Dilutions in							
	Saline		Serum		Saline		Serum	
1 3	++++	++++	++++	++++	++	—	++++	—
1 6	++++	++++	++++	++++	++	—	++++	—
1 12	++++	++++	++++	++++	+	—	++++	—
1 24	++++	++++	++++	++++	±	—	++++	—
1 48	++++	++++	++++	+++	—	—	++++	—
1 96	++++	++++	++++	++	—	—	+++	—
1 192	++++	+++	++++	++	—	—	+++	—
1 384	++++	+++	++++	+	—	—	++	—
1 768	+++	++	++++	±	—	—	++	—
1 1536	+++	+	++++	—	—	—	++	—
1 3072	++	+	+++	—	—	—	+	—
1 6144	—	—	++	—	—	—	+	—
1 12288	—	—	++	—	—	—	±	—
1 24576	—	—	+	—	—	—	—	—
1 49000	—	—	±	—	—	—	—	—
0	—	—	—	—	—	—	—	—

a matter of fact the titer against the A_1 cells is increased to 49000 The titer against the A_2 cells on the other hand is reduced to 768 (Part I c and d)

In the second part of table 10 serum Uge was neutralized by the addition of A and B substances resulting in the complete elimination of agglutinins for A cells and the almost complete neutralization of the agglutinin anti A_1 However if normal serum is used as a diluent cells of group A_1 again become strongly agglutinated in contrast to the A cells which remain completely negative

Obviously therefore, we are again dealing with an A_1 antibody of considerable potency and specificity as in the case of Mrs Bong Sera of the type described could be used very well for the differential diagnosis of A_1 and A cells with better results than most of the available commercial sera which have been prepared chiefly by the absorption of B serum with A cells

It has been known for quite some time that there is a group of A cells which

tially increased by this method * (3) The addition of 2 parts of AB substances to 1 part serum Wall results in a considerable decrease of both anti A and anti B titers in saline solution though the neutralization is not a complete one (4) Serum Wall neutralized in the way described agglutinates A_1 and B cells almost to the same extent as non neutralized serum provided all dilutions are made in normal human serum Examination of several other sera of this type revealed essentially the same results although sometimes the agglutination of A_1 and B cells by neutralized serum diluted in normal human serum did not equal the strength of the non neutralized preparation diluted in normal human serum It should also be mentioned that these immune sera did not lend themselves as readily to complement fixation as did the serum of Mrs Bong

TABLE 9—*Demonstration of Immune Isoantibodies Anti A and Anti B in the Serum of a Patient Following Transfusion with Conditioned O Blood and Plasma*

Serum Wall Group O	Blood Cells							
	Part I Not Neutralized				Part II Neutralized 1 3			
	A_1	B	A	B	A	B	A	B
	All dilutions in							
	Saline		Serum		Saline		Serum	
1 6	++++	++++	++++	++++	+++	++	++++	++++
1 12	++++	++++	++++	++++	+	+	++++	++++
1 24	++++	++++	++++	++++	+	±	++++	++++
1 48	++++	++++	++++	++++	±	—	++++	+++
1 96	++++	+++	++++	++++	±	—	++++	+++
1 192	+++	++	++++	++	—	—	++++	++
1 384	+++	+	++++	+	—	—	+++	++
1 768	+	±	+++	±	—	—	++	+
1 1536	±	—	++	±	—	—	++	—
1 3072	—	—	+	—	—	—	++	—
1 6144	—	—	±	—	—	—	+	—
1 12288	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—

Inasmuch as the anti A antibody occurring in the serum of patient Mrs Bong as demonstrated in table 8 was essentially an anti A_1 antibody, sera of other patients who had received pooled plasma or O blood conditioned with A and B specific substances were examined with regard to their content in isoagglutinins anti A_1 and anti A As an example serum of patient Uge who had received one transfusion with O blood conditioned with A and B substances was tested as follows

Decreasing dilutions of serum Uge group B (volume 0 2 cc) were mixed with 0 2 cc of 2 per cent cell suspensions A_1 and A_2 respectively In Part I the native serum was used in Part II, the serum was neutralized by the addition of 2 parts of

* The increase in the titers of immune antibodies by replacing saline solution with normal serum or similar diluents has been described by Boorman and Dodd as well as by Wiener

SUMMARY

The isoantibodies anti A and anti B which are described differ in several respects from those occurring in normal human serum This type of antibody has first been observed in the serum of an Rh negative woman who exhibited a history of erythroblastosis Her husband belonged to the subtype Rh_1 and to the blood group A The patient s serum completely neutralized with A and B substances still agglutinated strongly the husband s cells provided normal human serum replaced physiological saline solution as a diluent for all dilutions The impression was thus created that an Rh blocking antibody was responsible for the agglutination observed It could be shown however, that the abnormal antibody present in this patient s serum was not an Rh antibody at all but instead an antibody directed against the A property This type of anti A antibody resembles the Rh blocking antibody in many respects It becomes manifest only if undiluted human serum is used as a diluent Surprisingly enough this antibody agglutinated cells of group A although the amount of AB substances added to the serum was sufficient to neutralize completely the isoagglutinin anti A under normal conditions in which saline solution is used as a diluent This anti A antibody therefore cannot be neutralized as easily as the normal isoagglutinin anti A For its neutralization much larger amounts of the blood group specific substances are apparently necessary The patient s serum fixed complement when mixed with material containing water soluble A substance in contrast to the normal isoantibody anti A which failed to do so The titer of isoantibodies found in the patient s serum upon titration in saline solution was not extensively high and as a matter of fact was average It is therefore felt that an extremely high titer is neither a necessary requirement nor proof of isoimmunization toward the A and B factors Another interesting characteristic of the peculiar anti A antibody occurring in our patient s serum was the fact that it was essentially an anti A_1 antibody The difference in agglutination between A_1 and A_2 cells respectively becomes manifest if normal serum is used as a diluent instead of saline solution This difference becomes even more marked after neutralization of the patient s serum with A and B substances

During the course of Mrs Bong s pregnancy the special anti A antibody described did not increase but rather decreased in strength However even after delivery the antibody was demonstrable for at least several weeks although we had no opportunity to examine the patient s serum further That one must be very careful in contributing any pathological significance to isoantibodies anti A or anti B even of the type described is evident from the fact that the patient was delivered of a perfectly normal baby belonging to the blood group O and being Rh negative Whether the difficulties experienced by the patient in previous pregnancies were due to sensitization toward the Rh factor or to the A factor cannot be decided

Antibodies anti A and anti B of the type reported were also found in the sera of patients who had received large amounts of pooled plasma or O blood conditioned with A and B specific substances Again the anti A antibody occurring in the serum of these patients was mainly directed against the A_1 property Under the experimental conditions described in this paper such a serum can be used for the differential diagnosis of the subgroups A_1 and A and constitutes a sensitive reagent for

could be considered an intermediate between the A_1 and the A_2 subgroups The best way to study the intermediate subgroups consists obviously in the use of a quantitative titration which would give the best understanding of the degree of agglutinability of the cells under investigation An experiment of that type is recorded in table 11

Decreasing dilutions of serum Kale group O (volume 0 1 cc) were mixed with 0 1 cc of 3 per cent blood cell suspensions of (a) A_1 cells (b) A cells of an intermediate subgroup (c) A_2 cells A serum dilution of 1 50 was prepared for the experiment by adding to 1 part of serum Kale 3 parts of AB substances (Sharp & Dohme) and 46 parts of normal human serum of Group A Further dilutions were made in undiluted normal serum of group A, and the cell suspensions were prepared by using normal serum of group A as a diluent instead of physiological saline solution The tubes were kept for 30 minutes at room temperature centrifuged and read for agglutination

TABLE 11 —*Determination of Sub Groups by Anti A Immune Antibody*

Serum Kale Group O	Cells		
	a A_1	b A	c A_2
1 50	++++	++	−
1 100	++++	++	−
1 200	++++	+	−
1 400	++++	+	−
1 800	++++	±	−
1 1600	+++	−	−
1 3200	++	−	−
0	−	−	−

The experiment recorded in table 11 shows definite differences between the 3 A cells used The A_1 cell is strongly agglutinated the A_2 cell is completely negative however there is an intermediate A cell which seemed to contain a small but definite amount of A_1 substance It is interesting to note that this intermediate cell was not agglutinated by a commercially available anti A_1 test serum and had been classified as an A_2 cell on that basis Other intermediate cells have been found which were more strongly agglutinated by the anti A_1 antibody than the cell mentioned in table 11 On the other hand there were some cells which contained only traces of A_1 substance On the basis of the anti A_1 test serum prepared by the method described in this presentation we should arrive at a different percentage distribution of the subgroups of A than accepted at the present time Of 400 specimens belonging to blood group A examined so far we would classify 75 per cent as A_1 cells 15 per cent as intermediate and only about 10 per cent as A_2 cells The A factor in connection with the B factor as it occurs in the AB group is weaker than the A factor occurring in the A group a fact which conforms to previous observations However further investigations are necessary in order to reach definite conclusions regarding the subgroups of A in blood cells of the group AB

SUMMARY

The isoantibodies anti A and anti B which are described differ in several respects from those occurring in normal human serum This type of antibody has first been observed in the serum of an Rh negative woman who exhibited a history of erythroblastosis Her husband belonged to the subtype Rh_1 and to the blood group A The patient s serum completely neutralized with A and B substances still agglutinated strongly the husband s cells provided normal human serum replaced physiological saline solution as a diluent for all dilutions The impression was thus created that an Rh blocking antibody was responsible for the agglutination observed It could be shown however that the abnormal antibody present in this patient s serum was not an Rh antibody at all but instead, an antibody directed against the A property This type of anti A antibody resembles the Rh blocking antibody in many respects It becomes manifest only if undiluted human serum is used as a diluent Surprisingly enough this antibody agglutinated cells of group A although the amount of AB substances added to the serum was sufficient to neutralize completely the isoagglutinin anti A under normal conditions in which saline solution is used as a diluent This anti A antibody therefore cannot be neutralized as easily as the normal isoagglutinin anti A For its neutralization much larger amounts of the blood group specific substances are apparently necessary The patient s serum fixed complement when mixed with material containing water soluble A substance in contrast to the normal isoantibody anti A which failed to do so The titer of isoantibodies found in the patient s serum upon titration in saline solution was not extensively high and as a matter of fact was average It is therefore felt that an extremely high titer is neither a necessary requirement nor proof of isoimmunization toward the A and B factors Another interesting characteristic of the peculiar anti A antibody occurring in our patient s serum was the fact that it was essentially an anti-A_1 antibody The difference in agglutination between A_1 and A_2 cells respectively becomes manifest if normal serum is used as a diluent instead of saline solution This difference becomes even more marked after neutralization of the patient s serum with A and B substances

During the course of Mrs Bong s pregnancy the special anti A antibody described did not increase but rather decreased in strength However even after delivery the antibody was demonstrable for at least several weeks although we had no opportunity to examine the patient s serum further That one must be very careful in contributing any pathological significance to isoantibodies anti A or anti B even of the type described is evident from the fact that the patient was delivered of a perfectly normal baby belonging to the blood group O and being Rh negative Whether the difficulties experienced by the patient in previous pregnancies were due to sensitization toward the Rh factor or to the A factor cannot be decided

Antibodies anti A and anti B of the type reported were also found in the sera of patients who had received large amounts of pooled plasma or O blood conditioned with A and B specific substances Again the anti A antibody occurring in the serum of these patients was mainly directed against the A_1 property Under the experimental conditions described in this paper such a serum can be used for the differential diagnosis of the subgroups A_1 and A and constitutes a sensitive reagent for

the recognition of the differences occurring within the A factor With the aid of such a serum only 10 per cent of A cells were found to belong to subgroup A_2 75 per cent to A_1 and 15 per cent were considered to be of the intermediate type No subgroups were found so far in human cells of group B

DISCUSSION

Dr Hill Dr Race let us have your discussion

Dr Race I should like to add my congratulations to the last speakers They have made a tremendous contribution and Dr Levin s work I already knew about but Dr Witebsky s paper is something very exciting and it sounds to m as if he has years of work ahead of him I hope he will be able to find the time to follow up the cases similar to the one of Mrs Bong I have a question which I should like to ask Dr Levine what he thinks of this idea as an explanation of the extraordinary difficulty in the production of the anti Rh wh n the pregnancy is h t rosp cific I should like to ask Dr Witebsky a question at the same tim I want to ask if h tried h ating Mrs Bong s serum which will destroy the agglutinin and did it still giv th curious effect you describ d?

Dr Davidsohn I would like to add to the remarks made by Dr Rac regarding the relation of the h t erospecific pregnancy in some of th cas s d scrib d by Dr L vine C rtainly the whole th ory approaches the problem of homosp cificity or h terosp cifi ity We found that only h t rosp cific pregnancy was con cerned in our tests That would b in agre m nt with th pr viously reported pap rs Dr Levin m n tioned about 24 or so cases

In connection with Dr Witebsky s remarkable paper we made an observation on s v ral cases that when we had anti Rh sera that were difficult to neutraliz trem ndous amounts of A and B substance had to be used before the anti A or anti B could be eliminated and at that tim we found that by testing at 3 temperatures 37 C room temperature and ice box temp rature the highest titre is usually at the lowest temperature In some of these cases w found the highest titre at body temperature and significantly low r titres at ice box temperature We think that this is a manifestation of som degree of immunization

Dr Hill Dr Levine is first to answer questions in regard to his paper

Dr Levine Of cours the statistical deviation of group incompatible matings in Rh negative mothers as opposed to random matings is not striking (25% and 35% respectively) Nevertheless my observation was confirmed by the British workers Actually there is a greater incidence of matings incompatible for A and B (35%) than for Rh (13%) Possibly this indicates a greater antigenicity of the Rh factor In my opinion an answer to this question must await the outcome of comprehensive studies with particular reference to the secretor non secretor variation of the A and B factors

Dr Race Why doesn t a double immunization occur when a heterospecific pregnancy of A and B and Rh occur?

Dr Levine I have no explanation to offer I can t understand why when there is a double incompata bility they both couldn t immuniz because the body is capable of responding to numerous antigens that may be injected It is for that reason that I have little faith in Dr Wiener s suggestion that the injection of typhoid vaccine may perhaps prevent isoimmunization by th Rh factor I myself would welcome an answer to this question Whether or not the secretor or nonsecretor plays a part in this I do not know

Dr Witebsky Dr Race e have tried to heat thos sera containing the 2 different types of antibodies but I must admit that our results were inconclusive so I don t care to comment on that Of course it would be expected that this second type of immune antibody would be more heat stabile than the normal isoag glutinin but we are so far unable to prove this Maybe some others will be able to do so As far as Dr Davidsohn s remarks are concerned I too feel that this antibody is certainly not a warm antibody but rather the antibody that work at room t mp rature and maybe a little bit better in the icebox We have done most of our exp rim nts though at room t mp rature because it was the most convenient thing to do but it is certainly not th warm antibody

Dr Levine I think that it will be of interest to recapitulate some of the early d velopments of the ge netics of the Rh Hr system In 1941 I had available large quantities of potent anti D (anti Rh_0) and anti C (anti Rh) and a weak anti-c (anti Hr) On testing all donors of the Blood Transfusion Association I described but did not name four types of reactions ith anti D and anti C But anti C and anti c gave only three types and because of the analogy with the MN relationship I applied the term anti Hr to what

is now known as anti-c It was at th ame time impli d that th genetic relationship of C-c was closer than that of C D

At th written request of Dr Wien r this material was made available to him and the schema of the reactions appear in his book and els where Wiener neglected th Hr factor in his th ory of multiple al leles Wh n the E factor (Rh) was found Wien r m t the situation by increasing the number of genes at the one locus As additional variants are found it will be necessary for Wien r to keep adding alleles assuming that he will adhere to his theory From the very b ginning I was confident that the multiple allele theory was premature because it did not take into account the Hr factor

It is of great interest that the British workers went along with Wiener until they found two sorts of sera which like M and N gave only three and not four types of reactions The important consideration is the quality of the sera available to Race and Taylor in each of their three papers in Nature (1943-1944) At first they studied the relation hip of anti D and anti-c Their second study was based on findings with anti D anti-c and the newly discovered anti E Now each of these sera give four types of reactions when studied in parallel with any of the other two If the three are studied together then eight types of reac tions are observed ($2^3 = 8$)

It so happened that anti-C was the last of these four antibodies found by Race and his co-workers In 1944 these workers for the first time observed the three types of reactions given by anti-C and anti-c As in the case of M and N a blood failing to react with both sera does not exist

Accordingly Fisher and Race abandoned the multiple allele theory and suggested linkage at three different loci of a particular chromosome This made it necessary for Fisher to predict the existence of two other Hr genes When anti-e was found by Mourant and anti-d by Diamond the linkage theory became a reality I had already referred to my application of the Johannsen formula of gene frequencies which lends powerful support to the linkage theory

So far as terminology is concerned it is advisable to adopt the Fisher Race system There seems to be no need for the use of R or Rh as genes However there can be no objection to the use of the Rh symbols for the phenotypes along with the CDE system In referring to an Rh_1 individual one may use the term DC with the understanding that no reference to capital letters is necessary In terms of the Fisher Race theory an Rh individual is either DcE given orally as D small c E or simply DE

Genetic usage requires that allelic genes at any one locus be given as variants of a particular arbitrary letter If there is another locus on the same chromosome variants of another letter or symbol must be used to define the several allelic genes In a sense the use of D is fortunate since anti D refers to the clinically most important i e the *d*iagnostic serum

Finally I should like to mention that geneticists generally were taken by surprise when confronted with the findings on the pathogenesis of erythroblastosis fetalis Superficially at least the geneticist is reminded of lethal factors to explain this form of selective fetal and neonatal morbidity It is obvious however that the affected infant must be heterozygous while lethal factors are effective only in the *homozygous form*

HEMOLYTIC Rh IMMUNE GLOBULINS EVIDENCE FOR A POSSIBLE THIRD ORDER OF ANTIBODIES INCAPABLE OF AGGLUTINATION OR BLOCKING

By JOSEPH M HILL M D SOL HABERMAN PH D, AND FRANCES JONES B A

THE possibility of a third order of reactivity of Rh antibodies characterized by their failure to act either as classical agglutinins or true blocking anti bodies has been suggested in earlier publications [1, 2] In this paper additional evidence is presented characterizing immune globulins of this third order of reactivity In such a characterization of antibodies or immune globulins we might bear in mind that antibodies may be classified and characterized according to their response to a variety of tests and procedures For example antibodies may be clas sified according to their specificity or their mode of action They have also been designated according to their stability under the influence of heat whether they occur naturally or only in response to given antigens the time of their response in respect to the immunization curve and in many other ways

In the Rh field the study of antibodies has been particularly interesting In itially studies were concerned chiefly with the specificity of the different anti bodies observed utilizing the classical method of agglutination of red cells in saline suspension For example in the original report of Levine and Stetson[3] in 1939 the irregular isoagglutinin which they described had a specificity of ap proximately 80 per cent which was suggestively close to the specificity of 84 per cent which Landsteiner and Wiener[4] found when their anti rhesus serum was used to test human erythrocytes Different specificities for various human anti Rh sera was noted in 1941 by Levine[5] when he described specificities of 73 per cent 85 per cent and 87 per cent when tested with random human red cells In the same year Landsteiner and Wiener[6] also noted human sera containing agglutinins for red cells having different specificities These and the subsequent studies of Rh antibody specificity have proved to be of utmost importance in the working out of the relationship of the different Rh antigens and in the formation of sub-type classifications However the fascinating story of the discovery and use of sera of different specificity to unravel the intricate relationships of the different Rh antigens has been told by Doctor Race in his paper today We are much more concerned here with properties of the Rh antibody affecting its mode of action

In the early work with the Rh antibody the classical agglutination test utiliz ing a saline suspension of red cells was employed It was early noted that demon stration of Rh antibodies in cases of erythroblastosis was often lacking Further more clinical correlation of severity of the disease with antibody titres was often very poor or completely lacking Intensive investigations to improve methods of Rh testing stimulated by the necessity to demonstrate agglutinins more con sistently in connection with transfusion incompatibility and erythroblastosis led

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to a very important advance in our knowledge of antibodies In 1944 the separate reports of Race and Wiener[8] described an incomplete or blocking antibody often present in cases of Rh isoimmunization which could specifically adsorb and completely saturate the Rh antigen of the test erythrocytes without causing apparent agglutination The blocking test as it was called by Wiener, took advantage of the fact that Rh positive cells treated with such sera would no longer agglutinate when anti Rh serum of known potency was added A somewhat different approach was made to this same problem by Diamond[9] when he found that some Rh antisera seemed to contain an inhibitor substance which resulted in a lowering of potency when other higher titred anti Rh sera were pooled with such sera The development of the blocking test technic was an important contribution because it enabled us to demonstrate clearly this new kind of antibody by a convenient and practical method Earlier workers had detected the presence of antibodies or antibody like material in sera having a similar effect but lacking the blocking test were unable to differentiate clearly and identify this antibody Eisenberg and Volk[10] for example used the term agglutinoid to designate what they considered a modified agglutinin possessing the power to combine with but not to flocculate the agglutinogen

The blocking technic of course provided an additional method for the detection of antibodies in cases of Rh isoimmunization In our experience the method proved somewhat disappointing for this purpose because it increased only slightly the percentage detection of antibodies in cases of erythroblastosis As a method of studying agglutination phenomena and for the characterization of antibodies however the blocking test has been exceedingly useful The evident exhibition of first stage antibody reaction without subsequent agglutination has led to the conclusion by many investigators that the blocking antibody is univalent in nature as opposed to the bivalent agglutinin These findings served to explain the zone phenomenon seen in some anti Rh sera and suggested that zone phenomena generally might be accounted for by the presence of blocking antibody We feel the importance of the blocking test and the blocking antibody which it determines should not be obscured by interpretations given to the results obtained with other and later methods which will also demonstrate antibodies of blocking type These methods such as Diamond s albumin test,[11] Wiener s conglutination test[1] etc are considerably less specific than the blocking method in that they determine not only the blocking antibody but also the classical (saline) agglutinin as well as a certain portion of what we are designating third order antibodies in this paper

In 1944 Chown[12] presented a method of Rh testing of remarkable simplicity and high sensitivity In this method a heavy suspension of red cells or whole blood is in contact with the typing serum within a capillary held at an angle of 45° The red cells gradually fall through the zone of the agglutinin containing serum and agglutinate into very clear bands and large aggregates as the serum acts in the presence of capillary forces We introduced Doctor Chown s technic into our laboratory as a routine procedure early in 1945 and have used it with growing enthusiasm ever since We were most interested when we found that this method

as we used it was capable of detecting antibodies which would not agglutinate erythrocytes in saline in the test tube Still more interesting was the fact that many sera which gave positive results with the blocking test would not agglutinate Rh positive red cells in the capillary (It should be noted here that in our use of the Chown method we have employed a saline diluted serum the usual ratio being 0 1 or 0 2 cc of original serum to 1 cc of saline solution) The meaning of this peculiar discrepancy of course did not become clear until later It is obvious however that Chown s test in addition to the blocking and classical agglutinating methods helped to characterize antibodies further

In 1945 Diamond and Abelson[14] described a slide test which proved to be a very sensitive test for recognition of antibodies and paved the way for the conglutination and albumin tests In these later methods the importance of the suspension medium or fluid components of the test were recognized By the use of albumin or serum for preparing erythrocyte suspensions zone phenomena and blocking effects were largely eliminated from the agglutination test for antibodies These methods not only detected the classical saline agglutinins and the antibodies determined by the blocking test but also we believe detected antibodies not shown by the other two earlier methods Unfortunately the term blocking antibody has been extended to cover all of the antibodies detected by these newer tests employing colloidal suspension media

An entirely different method for the specific detection of antibodies was the use of anti human globulin serum as described by Coombs Mourant and Race [15] This new approach shifts the emphasis from the specificity of agglutination to the more basic phenomenon of specificity of antibody adsorption As a result the detection of antibodies does not depend upon their ability to function as agglutinins or by hapten saturation (blocking) but solely by their ability specifically to adsorb on the test erythrocytes The anti human globulin serum acts by developing an observable agglutination of red cells which retained the specifically adsorbed antibody (human immune globulin) The specificity of the test is determined by the choice of the proper antigen in this case known Rh positive erythrocytes For convenience we have used the term developing test in referring to this method of Coombs Mourant and Race[15] because it develops an observable reaction in a manner analogous to the development of a photographic image This test promised to be a particularly useful serologic tool to demonstrate any antibody for which a suitable specific antigen could be provided which would produce visible clumping

Coombs Mourant and Race found that the treatment of an anti C serum with heat (56 C) caused it to become nonreactive to the agglutinating blocking and conglutinating tests while its ability to adsorb as demonstrated by the developing test was still present These workers considered the method a more sensitive technic for the detection of weak and incomplete (blocking) antibodies This method of antibody detection was added to our routine procedures shortly after its publication As in the case of the Chown capillary technic we found interesting discrepancies in the saline agglutinin and blocking antibody titres as compared to the new method As reported in our earlier papers [1] cases were observed in which antibody titres as high as 1/2048 were found by the developing (Coombs) test

while the agglutinating titre (1/2) and blocking titre were minimal Extensive experience with the developing test on a large routine service made us feel confident that such discrepancies were well beyond the 1 or 2 tube greater sensitivity of this method On the basis of such findings with the addition of some experimental results we suggested the possibility of a third order of reactivity of immune globulins

Since these preliminary studies strongly suggested that the Rh antibody could exist in a form incapable of agglutination or blocking and yet gave evidence of its antibody nature by specific adsorption and hemolytic activity, further investigations were undertaken These were concerned with (1) the establishment of the validity and accuracy of the new methods such as developing test titrations and hemolysis quantitation (2) demonstration of antibody characteristics exhibited by the proposed third order immune globulins such as specific adsorption and hemolysis and (3) demonstration of differences from previously described antibodies which characterized the proposed new group of third order antibodies *

METHODS

Agglutination Studies of agglutination were carried out by the several methods described below All antibodies were titrated by serial dilution For routine purposes 1 cc serologic pipets calibrated to 0 01 cc were used to prepare the serum dilutions One drop of each dilution was placed in 7 mm (inside diameter) tubes by means of a capillary pipet with a tip approximately 1 mm in diameter In every instance this transference of a drop of serum was started at the highest dilutions to avoid error A similar capillary pipet was used to add 1 drop of a 2 per cent suspension of the test erythrocytes (suspension of 3 or 4 Rh positive bloods including different sub groups) After thorough mixing of the cells and serum the tubes were placed in a water bath at 37 C for 1 hour The tubes were then centrifuged at 750 r p m for 1 minute and observed for agglutination macroscopically and microscopically In the case of very high titres (1 10 000 or over) the routine technic was checked by a more accurate method of dilution in which several Kahn type pipets were used per test to avoid carry over of antibodies

Special Agglutination Tests Four tests namely the Diamond slide and albumin tests the Wiener conglutination method and the Chown capillary test were used to effect agglutination when blocking antibodies or zone phenomena interfered with agglutination by the standard test tube technic These tests were used as described by the authors

Blocking Test The method described by Wiener was employed to show the presence of blocking antibodies However after the observation was made as originally recommended we have obtained a sharper differentiation in the titration of these antibodies by centrifuging the tubes and again observing for agglutination

Developing Test To develop observable agglutination of otherwise non agglutinating Rh antibodies and immune globulin or cryptagglutinoids anti human globulin serum was used as originally described by Coombs Mourant and Race In this method one drop of the serum to be tested was mixed with one drop of a 2 per cent suspension of type O Rh positive erythrocytes in a Kahn type tube This was incubated for one hour at 37°C and observed for agglutination If no clumping was observed the cells were washed three times in saline solution by centrifugation to remove unadsorbed globulins After the third wash 1 drop of anti human globulin serum was added to the sedimented cells after the

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Recently we have proposed the term cryptagglutinoid to designate the antibody which neither blocks nor agglutinates saline suspensions of erythrocytes [14] The manuscript has been changed by substitution of this new term for th older and less descriptive designation used in the original presentation of this paper

saline had been removed The cells were suspended and incubated for one hour at 37 C At the end of this second incubation the tube was centrifuged at 500 r p m for one minute and observed for clumping Agglutination indicated the presence of cryptagglutinoids These tests were performed routinely on all cases of jaundice of the newborn and on all Rh negative mothers and their children

Hemolytic Activity of Rh Serum as Demonstrated in Vitro Three tenths cc of anti Rh serum was added to 3 cc of heparinized freshly drawn Rh positive whole blood All the blood used in the experiment was drawn into a dry 50 cc syringe through a No 19 needle and after the removal of the needle was placed in a sterile 125 cc flask containing 0 2 cc of sterile heparin solution This was done in order to keep the mechanically produced hemolysis to a minimum and also to eliminate any possible hemolytic effect due to bacterial contamination A set of 6 tubes was used for the test and 6 for the controls As a rule serum of the highest available titre was used The final effective titres were as indicated in the results of the experiment The reagent blank and a suitable control were set up in order to obtain the net hemolysis due to the Rh serum alone For the first blank 0 3 cc of anti Rh serum and 3 cc normal saline were used to make the final volume equivalent to that in the test This blank represented the hemoglobin present in the anti Rh serum which was used in the experiment The second blank consisting of 3 cc of saline and 0 3 cc normal serum was set up to measure the amount of hemoglobin present in the normal serum required in the controls

Since normal heparinized blood becomes slowly and progressively hemolysed on standing in vitro it was necessary to use a control as follows to 3 cc of the same heparinized blood which was used in the experiment was added 0 3 cc of the normal serum whose hemoglobin was determined in the second blank

For the test 3 cc of the heparinized blood were immediately added to each of 6 sterile tubes containing 0 3 cc of anti Rh serum For the control set 6 similar sterile tubes were used containing 0 3 cc of normal serum in place of the anti Rh serum A tube from each of the series was immediately centrifuged and the supernatant cell free serum was analyzed for its hemoglobin content A modification of the Bing and Baker[14] test for hemoglobin was used The remaining tubes of both test and control were incubated at 37 5 C and at 2, 6 20 30 and 48 hours a tube from each series was removed centrifuged and the hemoglobin content of the supernatant plasma determined A Lumetron 2-cell photoelectric colorimeter using narrow band filters and a high sensitivity double reflecting galvanometer was used to determine the color produced by the benzidine reaction

Net hemolysis was calculated in mg of hemoglobin from the formula $(H_t - b_t) - (H - b) =$ net hemolysis

Where

H = mg per cent of hemoglobin in supernatant of tubes containing blood and antiserum (experiment)

H = mg per cent of hemoglobin in supernatant of tubes containing blood and normal serum

b_t = mg per cent of hemoglobin in first blank (Rh serum + saline)

b = mg per cent of hemoglobin in second blank (normal serum + saline)

Electrophoretic Studies Electrophoretic analysis and fractionations were made in a Tiselius apparatus using the optical arrangement of Longsworth for observing boundaries The instrument used was one assembled by Dr Dan H Moore of Columbia University Patterns were made on 11 cc aliquots of serum in a double sectioned cell at 0 5 C Prior to analysis the serum was diluted 1 4 with 0 02 M sodium phosphate pH 7 4 containing 0 85 per cent sodium chloride and then dialyzed for 24 hours (at ice box temperature) against a large volume of buffer prepared in the same way This same buffer was used to fill the apparatus

After electrophoresis the components of the serum were distributed in layers throughout the length of both arms of the cell Separation of these fractions was accomplished by carefully noting the position of the boundaries then removing the successive layers by means of capillary pipets care being taken to avoid disturbing the boundaries

* In later experiments to be reported elsewhere guinea pig complement was also added to both the test and control series in order to provide an excess of complement and produce maximum hemolysis By this technique much more complete hemolysis was observed for example approximately 1 gram of hemoglobin per 100 cc of supernatant plasma was attained in some instances

RESULTS

The application of the agglutination, blocking and developing tests to the routine services of the blood bank and maternity unit, as well as to the experimental production of hyperimmune anti Rh sera resulted in the demonstration of antibody patterns that are deemed worthy of report These results, along with the studies on the electrophoretic separation of Rh immune globulins and the quantitative demonstration of hemolytic activity of the agglutinating and crypt-agglutinoid antibodies, are described below

Case 1 A type O Rh negative female gave birth to a severely jaundiced infant The past history showed that the mother had received 1 transfusion and had had 2 miscarriages The type O Rh (D) positive infant s erythrocytes gave a 4+ developing test The antibody pattern presented in table 1 was found on examination of the mother s serum on the day of birth In this instance we have completely negative agglutination and blocking tests Nevertheless by the developing test this serum was able to sensitize Rh (D) positive erythrocytes up to a dilution of 1/512

TABLE 1—*Serum Antibody Pattern from Mothers of Erythroblastosis Cases*

	Antibody Titre												
	1	2	4	8	16	32	64	128	256	512	1024	2048	4096
Case 1													
Aggt	−	−	−	−	−	−	−	−	−	−	−		
Block	−	−	−	−	−	−	−	−	−	−	−		
Develop	++++	+++	+++	++	++	++	++	++	+	+	−		
Case 2													
Aggt	+++	+	−	−	−	−	−	−	−	−	−	−	−
Block	++++	++++	++++	+++	+	±	−	−	−	−	−	−	−
Develop	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	+	+	−
Case 3													
Aggt	+	−	−	−	−	−	−	−	−	−			
Block	+++	++	+	±	−	−	−	−	−	−			
Develop	++++	++++	++++	++++	+++	+++	+++	+	+	−			

Case 2 A type O Rh negative mother delivered a stillborn macerated infant at an estimated 8 month gestation Two years before this pregnancy the mother had received 2 blood transfusions following an appendectomy The serum collected on the day of delivery showed the antibody pattern presented in table 1 The condition of the macerated infant precluded any studies of the antibodies in its serum In this case antibodies could be demonstrated by all 3 methods The results suggested a mixture of anti bodies The titre by the developing test seemed sufficiently greater (6 tubes) to definitely suggest an excess of antibodies not revealed by the other 2 methods

Case 3 The mother of a severely jaundiced infant whose erythrocytes gave a 4 plus developing test presented an unusual past history Her first child died shortly after birth of a disease supposedly erythroblastosis During this pregnancy the mother had received small intramuscular injections of the husband s blood with the purpose of preventing erythroblastosis Shortly before delivery the patient was transferred from another city to Baylor Hospital The pregnancy terminated in the birth of an extremely jaundiced infant which was treated with Rh negative blood transfusions and subsequently recovered At birth the infant s erythrocytes gave a 4 plus developing test The eluate from the cord blood showed neither agglutinins nor blocking antibodies but a titre of 1/16 was found by the developing test The mother s serum taken on the day of birth showed the antibody pattern given in table 1 In this case the titre of antibodies demonstrated by the developing test is greatly in excess of the blocking

antibody while agglutinins are practically absent Apparently here the antibodies are a mixture of the blocking variety and cryptagglutinoids

Case 4 The Rh negative patient was admitted to the hospital for a possible pelvic abscess following a laparotomy and because of a persistently low erythrocyte count (2 900 000/c m m) The patient had had 2 caesarian deliveries with blood transfusions from th husband each tim During th hospital stay a transfusion was ordered The husband offered himself as the donor Wh n it was found that he was Rh positive the antibody pattern of h r serum was studied The results pres nted in table 2 demonstrate that isoimmunization had occurred Subsequent Rh negative transfusions were given without incident This antibody pattern shows a low titre of agglutinins no blocking antibodies and a fairly high titre of immune globulins or cryptagglutinoids Th re is also a p culiar zon effect in th developing test for which we have not found an adequate explanation

TABLE 2 –*Serum Antibody Pattern from Mothers of Erythroblastosis Cases*

	Antibody tit e									
	1	2	4	8	16	32	64	128	256	512
Case 4										
Aggt	+	+	−	−	−	−	−	−	−	−
Block	−	−	−	−	−	−	−	−	−	−
Develop	+	+	+	+++	++	++	+	+	+	−
Case 5										
Aggt	+++	±	±	−	−	−	−	−		
Block	+	++	+++	++	++	+	−	−		
Develop	++++	++++	++++	++++	+++	++	±	−		
Case 6										
Aggt	++++	++++	++++	++++	+++	++	+	±	−	
Block	−	−	−	−	−	−	−	−	−	
Develop	++++	++++	++++	+++	+++	++	+	+	−	
Case 6										
Aggt	+++	++	++	+	−	−				
Block	−	−	−	−	−	−				
Develop	+++	+++	++	+	±	−				

Case 5 An Rh negative mother gave birth to a severely jaundiced infant Six years before this pregnancy the mother had received 3 transfusions There was no history of previous pregnancies The infant s erythrocytes gave a 4 plus reaction to the developing test The antibody pattern in the maternal serum on the day of birth is presented in table 2 This pattern illustrates the situation where the developing test parallels the blocking test apparently showing that only the blocking antibody is responsible for the titre of 1 64 shown Since this is a plus minus reaction the results are practically identical We feel that this is the case when pure agglutinating or blocking antibodies are demonstrated by the anti human globulin technic A titre 1 tube higher is frequently found as compared to the agglutinating or blocking tests

Case 6 An Rh negati e mother gave birth to a slightly jaundiced infant whose erythrocytes gave a 4 plus developing test The mother had had one previous child who was living and v ell The serums collected on the day of birth and 1 month later showed the antibody patterns presented in table 2 The similarity of the antibody patterns of these studies shov s a relatively similar sensitivity of the developing test and agglutination test hen only 1 type of antibody is present It is probable that these sera had only the agglutinin present

Antibody Patterns Studied during Deliberate Hyperisoimmunization for Rh Serum Production In addition to the above cases another group should be described because of significant antibody responses and patterns This group consisted of individuals who had received small doses of Rh positive cells with the deliberate intention of producing high titre Rh typing serums [17] With these individuals antibody patterns and variations could be very closely followed in relation to administration of the Rh antigen

Table 3 —*Mrs R W Titre of Anti Rh Agglutinins Produced by Injection of Rh Positive Blood into Previously Isoimmunized Woman*

Birth of Icteric Ch 11-4 29 43 Days Since Immunization Started	Blood Injected cc	Agg t	Block	Developing
		1/1280	*	*
		1/1280	*	*
		1/1024	*	*
		1/64	*	*
1	2			
4		1/128	—	
7		1/512	—	*
	5	$1/640 Rh_1$		
		$1/1280 R_2$		
11		1/2560	—	*
20		1/4096	—	
25	5	1/4096	—	*
31	5	1/4096	—	*
32	5	1/8192	1/16	*
195		1/32 000	—	1/128 000
224		1/1024	—	1/1024
29[illegible]		1/256	1/4	1/2048
408		1/2048	1/32	1/32 000
409	5 i v			
	1 i m			
410		1/1024	1/32	1/1024
411	5	1/1024	1/8	1/1024
414	1 i v			
	1 i m			
		1/1024	1/8	1/2048
415		1/2000	1/8	1/2000
416		1/4000	1/2	1/4000

The tests were not done in the cases marked with asterisks in tables 3 and 4

The first of this group Mrs R W has been described in detail in an earlier publication [17] She was the mother of a severely icteric second child who recovered with the administration of multiple Rh negative transfusions The original titre of the mother s serum was 1/1280 by the agglutination reaction Blocking and developing tests were not available at that time In table 3 the schedule of intravenous injections of Rh positive blood is given together with the time intervals and

antibody response The various long time intervals were du- in part to the itinerant habits of the volunteer donor

The second individual to be hyperimmunized Mrs O M was the mother of a severely icteric child who had recovered after treatment with multiple Rh negative transfusions The original titre of the mother s serum was 1/256 by the agglutination reaction The results of the intentional isoimmunization program are presented in figure 1 The program was started in April 1946 and was discontinued temporarily by an automobile accident to the volunteer Several months later the isoimmunization was undertaken again In this case the agglutination reaction

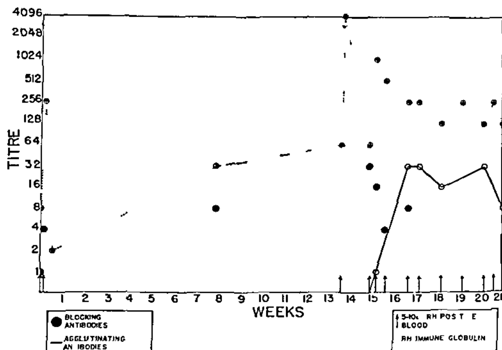


FIG 1 ANTIBODY PATTERN PRODUCED BY INJECTION OF Rh POSITIVE BLOOD INTO A PREVIOUSLY ISOIMMUNIZED WOMAN

failed to reach a titre suitable for the production of typing serum However the *antibody pattern obtained is of considerable interest*

The third of this group who showed very significant antibody patterns during a course of injections of Rh positive blood to produce Rh testing serum was a male patient Mr P D admitted in September 1943 for the surgical treatment of polyposis of the colon and rectum A colectomy was p-rformed and later further surgery was done to remove the remaining rectum and anus During this treatment the patient *had been given multiple transfusions which finally resulted in a severe* transfusion reaction It was found that the patient was Rh negative and had an anti Rh′(C) titre of 1/128 Subsequent transfusions of Rh negative blood were uneventful In March 1946 the patient volunteered for the isoimmunization program The results presented in figure 2 are remarkable for the extremely high titres

by the developing test and especially at those points where no antibodies could be demonstrated by the agglutination or blocking technic

Study of Antibody Patterns during Deterioration During the isoimmunization of Case 1 several blood collections were made for the preparation of anti Rh typing serum The third collection of 250 cc yielded a serum with an agglutination titre

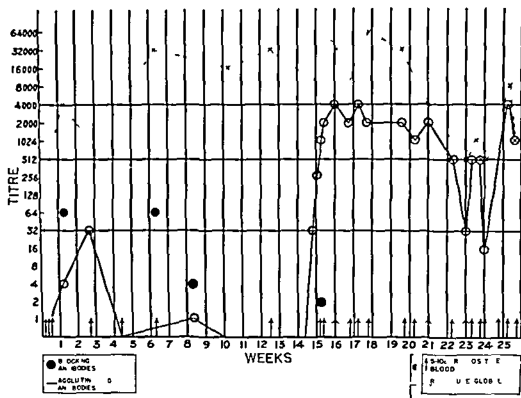


FIG 2 TITRE OF ANTI Rh AGGLUTININS PRODUCED BY INJECTION OF Rh POSITIVE BLOOD INTO A MAN PREVIOUSLY SENSITIZED BY TRANSFUSION

TABLE 4 —*Deterioration of a Labile Serum Stored at −20 F*

Date	Agglutination	Blocking	Develop
8-7-45	1/8192	1/16	
8-9-45	1/2048	1/64	*
4-1-46	1/1280		1/2048
4-10-46	1/1024	1/256	1/2048
4-17-46	1/2 weak	1/4	1/2048

of 1/8192 and a blocking reaction of 1/16 The serum was stored in a freezing unit at 15° F Within two days the agglutination titre began to fall It was decided not to use this serum for typing purposes From time to time the serum was examined for the antibody pattern as this deterioration of agglutinins progressed The results as presented in table 4 represent the antibody pattern found during the deterioration of this labile serum

In figure 3 is shown the *Electrophoresis* pattern of the serum of Mr P D of the serum production program when the blocking titre was 1/2 and the agglutination and developing titres were 1/4000 The electrophoretically separated albumin and globulin fractions were tested for the antibody pattern to determine the location of the antibodies in the serum fractions These were found in the gamma globulin



FIG 3 ELECTROPHORETIC PATTERN DAVISON SERUM

The studies of the hemolytic effects of Rh immune globulins are presented in graph form In figure 4 is presented the comparison of the benzidine dihydrochloride modification of the method of Bing and Baker[16] as used by Hill and Haberman[2] and the iron thiocyanate method as described by Moore[18] for the determination of hemoglobin The results of these 2 methods were so nearly identical that it was decided to use only the benzidine dihydrochloride technic in the later experiments The third graph in figure 4 shows the comparison between the use of saline and the use of normal serum in the controls to replace the anti Rh serum being tested for hemolytic activity No significant difference was found between these 2 methods of preparing controls

To prove the specificity of the hemolytic activity of anti Rh immune globulins, the experiments were repeated on both Rh positive and Rh negative cells These results are given in figure 5 In this experiment the hemolytic action of the crypt-

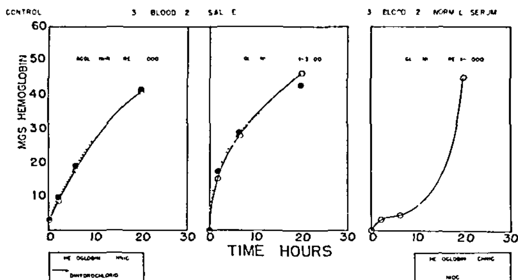


Fig 4 Hemolytic Action of Rh Antibodies on Rh Positive Heparinized Whole Blood

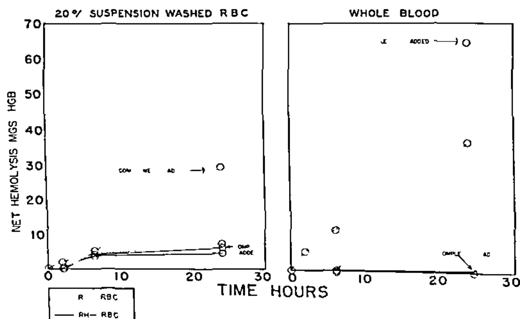


Fig 5 Comparison of Effect on Rh+ and Rh— R B C by Cryptagglutinoids

agglutinoid antibody is studied with reference to complement In the first graph the Rh positive and Rh negative erythrocytes were washed 3 times in saline by centrifugation and diluted to a 20 per cent suspension To one aliquot of these cells complement was added (2 units) and no complement was added to the remainder

In the figure it can be seen that no significant hemolysis as compared to the controls was observed against Rh negative cells Also, very little hemolysis occurred when no complement was added to Rh positive saline suspensions However, when complement was added to Rh positive saline suspensions significant net hemolysis occurred When the above experiments were repeated with Rh positive and Rh negative whole blood similar results were obtained The greatest hemolysis was produced when complement was added to the mixture of Rh positive whole blood and anti Rh antibodies When complement was not added to the Rh positive blood significant hemolysis occurred due to the complement already present in the fresh human blood used in the experiment The Rh negative blood showed no net hemolysis when treated with the anti Rh serum under the conditions of these experiments

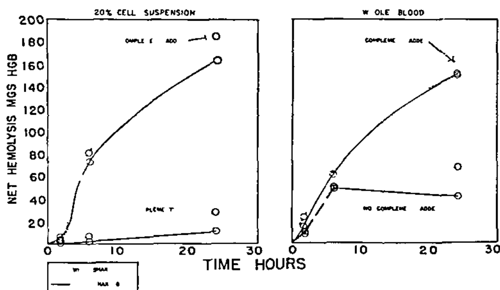


FIG 6 HEMOLYTIC ACTIVITY OF CRYPTAGGLUTINOIDS

Further studies on the hemolytic capabilities of the cryptagglutinoid anti body were made in the same manner as those presented in figure 5 introducing the effect of shaking to the mixtures of Rh positive blood complement and anti Rh serum The tests for the effects of shaking were performed in 25 cc Erlenmeyer flasks which were mounted on a rotary table The rotary travelled at a speed of 100 r p m and caused the blood mixture in the flask to mix gently The results pre sented in figure 6 show that the hemolytic effect of the Rh cryptagglutinoid was enhanced by complement and shaking

To compare the effect of different titres of anti Rh agglutinins and cryptagglu tinoids on the same Rh positive cells (Rh_0 or CDe) the following experiment was done A pure agglutinating serum (original titre 1/32 000) was added to 3 tubes containing Rh_0' (CDe) blood with the intention of producing a final titre of 1/1000 1/100 and 1/10 However after the preparation of the dilutions a final titration showed only 1/500 1/25 and 1/2 5 To a similar series of Rh_0' (CDe) blood was

added a pure cryptagglutinoid serum (original titre 1/16 000) In this case final titres of 1/2000 1/100 and 1/10 resulted It was hoped that examination of the hemolytic activity of the preparations described would give some evidence of the sensitivity of the developing test titration and establish the validity of titration by this method These mixtures were then studied in the same manner as described in the presentation of methods The results show that as the titres of both the agglutinin and the cryptagglutinoid antibody were increased, the hemolytic effect became more marked with relatively good correlation of titre and hemolysis These results presented in figure 7 indicated that the developing test was not merely a supersensitive technic but determined titres of cryptagglutinoid antibodies

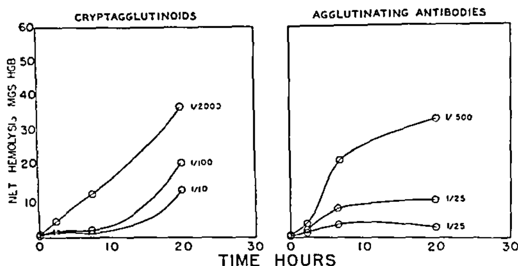


FIG 7 COMPARISON OF EFFECT OF AGGLUTINATING AND CRYPTAGGLUTINOIDS ON Rh+ R B C AT DIFFERENT TITRES

DISCUSSION

It would seem apparent that the forces involved in antigen antibody reactions are so closely linked to physical chemistry that attempts to represent pictorially these activities on the basis of the knowledge at hand is pure speculation Such pictorial representations as have been given in the recent past for the blocking and agglutinating antibodies seemed adequate for a short period of time However, the Diamond slide and albumin tests and the Wiener serum suspension tests quickly demonstrated its inadequacy while the new technic of Coombs et al [15] opened up new possibilities for study of antibodies as herein reported

When the agglutination reaction as performed in the test tube is used as the index of Rh sensitization it was found that many cases of clinical isoimmunization could not be explained due to the failure to demonstrate the classical agglutinin Later the discovery of the blocking antibody resulted in the demonstration of an antibody possibly incomplete with respect to valence In this case the antibody was capable of being adsorbed without producing agglutination in the test tube The obvious explanation appeared to be purely one of valence or incompleteness of the antibody molecule However the newer evidence found by Diamond and

Denton[11] by varying the suspension medium of the test Rh positive erythrocytes showed that the blocking antibody could be made to produce agglutination by substituting colloids such as serum and albumin for saline solution The reaction of Wiener using neutral serum as the erythrocyte suspension medium for the tube test produces the same results It would seem then that the valence of antibodies as originally propounded by Marrack[19] and Heidelberger and Kendall[20] and pictorially represented by Wiener for the Rh complex does not entirely explain the findings in the Rh problem Although the principle of valence may yet explain the blocking antibody it remains to be shown that such a concept explains the cryptagglutinoid antibody

From investigations in the present paper and in previous reports [1] it appears that the antibody demonstrable by the developing test differs from the classical agglutinin and the blocking antibody This antibody is capable of specific adsorption *without causing agglutination or blocking* However when such antibodies are tested by the albumin and serum methods agglutination occurs but in a somewhat lower titre than with the developing test In one case clearly showing antibodies by the developing test no agglutination could be detected by the conglutination method * It would seem then that the problem of antibody reactivity rather than valence alone is concerned On this basis 3 orders of antibody reactivity may be considered First specific adsorption with subsequent agglutination (classical agglutinins) second specific adsorption with saturation of the antigen and no agglutination (blocking) and third specific adsorption without evident saturation of the antigen and without agglutination (cryptagglutinoids)

Evidence for such a third order of Rh antibodies differing from the agglutinating and blocking types has been presented The evidence has been offered in the following catagories (1) Studies of antibody patterns in the serum of mothers of erythroblastotic children (2) antibody patterns found during deliberate hyperisoimmunization for the production of Rh typing serum (3) investigation of changes in antibody characteristics during deterioration in vitro over a period of time (4) the demonstration of the hemolytic action of the Rh antibodies, especially the third order or cryptagglutinoid type to establish their antibody nature and (5) studes of electrophoretic separations of Rh antisera with a view to determining whether the agglutinating and cryptagglutinoid antibodies were found in different protein fractions

In examination of the sera obtained from the mothers of erythroblastotic children it was found that the test tube agglutination method failed to detect many instances of isoimmunization Case 1 of this series demonstrates such an instance In this case the blocking test failed to reveal the antibodies However when the developing test was used a hidden antibody titre of 1/512 was found In the second case the blocking titre was 1/32 with a weak agglutinin present at 1/2 dilution The developing test showed a titre of 1/2048 a 6 tube difference between it and the blocking test and a 10 tube difference above the agglutination reaction The antibody pattern of Case 3 also shows these wide differences between the three

* Since presentation of this paper 4 clear cut examples of cryptagglutinoid antibodies demonstrable with the developing test but negative with the albumin or conglutination tests have been studied

titration methods In Cases 4 and 6 are presented instances where the blocking antibody was not present In Case 4 a 7 tube difference was found between agglutination and developing reactions The weak developing reaction at a titre of 1/4 (zone effect) found in this case is probably due to some carry over of nonspecific globulins which could neutralize the developing serum In Case 6 the cryptagglutinoid titre and the agglutination titre were almost the same, and it is believed that sera of this type represent mostly agglutinin with very little or, more likely, no third order antibody

The use of the anti human globulin serum of Coombs Mourant and Race as a developing test not only detects the third order (cryptagglutinoid) antibody but also yields visible agglutination when the blocking effect is found and when agglutinins are present as questionable or weak reactors When this antibody pattern approach was applied routinely to the study of sera from the maternity service an improved degree of correlation was found between titre of antibody and severity of erythroblastosis

In the examination of the data concerned with deliberate increase of antibody titres to produce testing sera it can be seen that the agglutinins were persistent in the case of Mrs R W However the blocking effect was transitory and never reached a significantly high titre, while the cryptagglutinoid antibody in many instances was much stronger than the agglutinin Also the antibody titres can be lowered by using different Rh subgroup cells in the isoimmunization series This change in titre resembles a laglike phase in the progress of antibody production At one time during the program the developing (cryptagglutinoid) titre reached 1/128,000 and agglutination was evident at 1/32,000 with saline suspension of erythrocytes It should be noted that as the immunization program progressed the cryptagglutinoid titre dropped and no longer exceeded the agglutinin titre This result seems to indicate that there is a degree of independence in the production of the antibodies studied

In the case of Mrs O M the blocking antibody was evident with some persistency However, when the frequency of blood injections were increased to 2 or 3 times per week the blocking antibodies disappeared and agglutinating antibodies became evident Throughout the stimulation period the cryptagglutinoid antibody exceeded the strength of the agglutinin or blocking antibody

In the case of Mr P D , it was found that several years after the original isoimmunization by transfusion a developing (cryptagglutinoid) titre of 1/256 persisted while the blocking and classical agglutination tests were entirely negative Five days after the initial injection of Rh positive blood, agglutinins and blocking antibodies appeared in the volunteer s serum In one series of tests in the study the volunteer s antibody pattern completely failed to show blocking antibodies or agglutinins although a very high titre of cryptagglutinoids (1/16 000) was observed When the Diamond albumin and Wiener serum tests were applied agglutination occurred at titres of 1/4096 and 1/1024 respectively suggesting that a part of the cryptagglutinoids were sufficiently reactive to be demonstrated by these methods In continued studies of this type similar results were observed Furthermore agglutination could be observed in Chown s capillary

test with many serums giving positive albumin tests However serums containing pure blocking antibodies only would not agglutinate Rh positive cells by this method The blocking antibody appeared at irregular intervals in the isoimmunization programs and rarely attained significant strength On the other hand the data presented shows that the cryptagglutinoid antibody appeared in the patient s serum first usually attained the highest titres, and persisted longer in the patient s blood stream

In reviewing the data on the deliberate hyperisoimmunization of the 3 individuals presented it can be seen that considerable variation occurred These differences in antibody patterns can be attributed to the individual s particular response to the repeated injection of Rh positive blood No apparent correlation could be made between the type of antibody and the phase of immunization although the cryptagglutinoid type of antibody persisted longer after antigenic stimuli ceased and could often be more easily increased during the active phase of stimulation However the cryptagglutinoid antibody often appeared first while agglutinins might or might not appear later On the other hand we had previously observed a case where agglutinin titres up to 1/100 000 were produced by the injection of Rh positive cells and at no time were blocking or cryptagglutinoid antibodies detected

The study of the labile serum collected from Mrs R W of the immunization program showed that the cryptagglutinoid titre remained constant while the agglutinin deteriorated rapidly The apparent increase in the blocking titre may have been due to the lability of the agglutinin

The specific nature of the adsorption of the Rh cryptagglutinoid antibody on Rh positive erythrocytes is strongly suggestive of the destructive role this antibody must play in sensitizing the red cells for their hemolysis in vivo so characteristic of Rh transfusion reactions and erythroblastosis Prior to our recent reports on hemolytic activity of Rh antibodies [1] [2] Diamond and Abelson[9] had noted hemolysis in doing their slide test In the quantative experiments described in this paper we have attempted to establish the hemolytic nature of the crypt agglutinoid antibody detected by the developing test It was considered important to demonstrate that the globulins shown to be specifically adsorbed on red cells were actually able to function as antibodies It was also found that complement was essential to their hemolytic action as in the case of the Rh agglutinin The amount of hemoglobin released in these experiments was small but significant because in each case without exception there was a definite net hemolysis as compared to controls In terms of the sensitive chemical technic employed however this magnitude of hemolysis was easy to detect quantitatively

Electrophoretic pattern studies of sera containing Rh agglutinins on the one hand and cryptagglutinoids on the other showed no significant variation from the normal Furthermore when separations were made by removing the protein fractions from the electrophoresis cell and titrations of each fraction performed it was found that both agglutinins and cryptagglutinoids were limited almost entirely to the gamma globulin These studies have not been sufficiently extensive to be considered other than preliminary in nature

SUMMARY AND CONCLUSIONS

We believe that sufficient evidence exists to justify 3 classes of Rh antibodies based on their reactivity These are the classical agglutinin the blocking antibody and the proposed cryptagglutinoid Sufficient distinction exists to retain the blocking antibody as determined by Wiener s original blocking test This antibody is further characterized by failure to act in Chown s capillary technic The identity of this antibody with its important role in reawakening investigation in this field of immunology should not be lost through inclusion in the broader group of those antibodies determined by the albumin test and similar methods The proposed third order antibody or cryptagglutinoid which is usually, but not always detected by these more inclusive tests and the capillary technic appears to be of great clinical importance because of the frequency with which it appears in significant titres Detection of isoimmunization and closer correlation of antibody titre with clinical severity of disease should be possible through study of this cryptagglutinoid antibody

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problem of the production of anti human globulin serum which we have called the developing s rum is not a simple one Production of the antibodies in the rabbit is not too difficult to obtain How ver the purification of this serum is the crux of the problem If red cells used to adsorb out the unwanted anti bodies are insufficiently washed or improperly washed some globulin will remain adsorbed on the red cells or in the saline used for the washing process When such a red cell concentrate is used there will be sufficient globulin present to neutralize a good deal of the anti human globulin factor Cons qu-ntly the titre avidity and sp-cificity of such a serum will be definitely lowered if not completely lost On the other hand if red cells are washed too frequently that is 8 9 or 10 tim s as a precautionary measure to eliminate the presence of serum or s rum proteins in the red cell pack the red c ll m mbran may be damaged and release hemoglobin which seems to be capable of acting som what as a globulin in af fecting the serum adversely We have found such hemoglobin to n utraliz som of the antibody and serum produced by overwashed cells have a titre that seems ad quate and an avidity sufficiently high but the sensitivity of the serum is greatly reduced giving poor or very weak reactions where 4 plus or strong reactions should have been anticipated We advise that the cells b washed 5 tim s in salin with all of the supernatant car fully removed after the last centrifugation and the adsorption carried on with equal quantities of serum and washed cells Frequently it will take as many as 5 or 6 such adsorptions before the serum is sufficiently purified We have found that adsorption in the icebox frequently removes the anti human globulin antibody by nonspecific adherence We prefer to do the adsorptions and purifica tion of developing serum at room temperature where such nonspecific adsorption of desired antibodies is at a minimum After completing the adsorption process it is wise to test the serum against some 50 bloods to be sure that all of the anti human red cell factors have been removed We have found that it usually takes more than a month of immunization using 2 or 3 inj ctions intravenously each week for a sufficiently high titre of anti human globulin antibody to b produced Use 1 cc intravenously for each inj-ction It is not unusual to have some of the rabbits fail to show human globulin antibodies of a sufficient high titre or avidity

We have used purified globulin or whole human serum for the production of the anti human globulin serum The commercially available immunizing globulins such as the typ- used in measles prevention should not be used as the antigenic stimulus for the production of developing serum

Dr Hill In answer to Dr Scudder s question concerning further physico-chemical studies I am sorry to say that we have not had the facilities to do ultracentrifugal studies and to determine sedimentation constants Our electrophoretic studies however must be considered preliminary in nature because of the brief period of time during which such studies have been made During these investigations we have consistently found all 3 orders of antibodies namely the classical agglutinins the blocking antibodies and the third order antibodies to be in the gamma globulin fraction In one case the gamma globulin was re run in the electrophoresis cell with results showing a single peak in the gamma globulin with the antibody titre present in the gamma globulin being identical to that found in the original separation Also tests for Rh antibodies which were run on the protein fractions remaining from the original separa tions after withdrawal of the gamma globulin failed to show evidence of Rh antibodies It is quite ob vious that we can draw no conclusions from such meager data and that many more experiments must be performed to determine this point One difficulty in doing work of this sort is the problem of having sufficient quantities of very high titred sera containing relatively pure classical agglutinins on the one hand or third order antibodies on the other We will of course be very interested to know what Dr Diamond and Dr Cohn will be able to discover in resp-ct to the molecular siz of these different forms of antibodies through use of the ultracentrifuge

In answer to Dr Levine s point concerning possible special symptomatology for infants having ad sorbed different types of antibodies that is blocking versus agglutinating I should like to state that we have not observed any correlation between the typ- of antibody and th clinical diseas Rather any correlation apparent has been related to the titre as shown by the developing test We feel that correlation of titre and clinical severity of the disease will be considerably improved by the use of such methods as Diamond s albumin test or through the use of any of the methods which are capable of detecting substantially all forms of antibodies Dr Waller s experience with the variability of response of different patients through stimulating doses of Rh positive cells is similar to our own Our experience leads us to believe that the most variable factor is the individual recipient rather than the method or the amount of blood given although we do have the impression that our somewhat larger doses when given

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DISCUSSION

Dr Uribe We are going to call now for a discussion of Drs Hill and Haberman s paper

Dr Ra e I should like to congratulate Dr Hill and Dr Haberman on their very fine work It s ems to me they have gone a long way toward making out a case for a third order It struck m that th storage experiment was particularly significant where the agglutinin and the blocking antibodies fell completely and there was no change in titre of the third order antibody Regarding terminology I think th analogy to the photograph is a heavy one and the developing test is a good name Certainly th nam rabbit anti human globulin serum which we use is quite impossible

Dr Robert K Waller I would like to present the last 2 cases we ran into One of them was a woman who was Rh negative and we immunized her with a small amount of Rh positive blood Initially she had an anti Rh agglutinating titre of roughly 1 4 or 1 8 after the first injection and then 14 days later the titre rose to approximately 400 or 500 We gave another injection of about 2 cc of blood and the titre rose to approximately 4 000 after 10 days Now we concluded if we could get it up to 4 coc we could get it up higher and injected about 5 cc of red cells and got a blocking antibody of 4 000 and a complete disappearance of agglutinins I tried to adsorb those blocking antibodies out by the m thod developed by Dr Levine and myself that is by adsorbing at zero temperature and we could not demonstrate any agglutinins with the exception of a small agglutinin titre of 1 4 That was the first case The second case was a mother who after the birth of her erythroblastotic child had a titre of approximately 1 500 We bled that woman and want to bleed her again after 4 months In the meantime she has not become pregnant again and has not received any transfusions and she only has blocking antibodies and no agglutinins I wonder whether you could offer an explanation of this

Dr Levine Dr Hill have you noticed any special symptomatology in these infants when they have blocking antibodies exclusively? I see that you did not do any tests with titration of the antibodies with the albumin test Now I first thought I had a third order of antibodies in the titration of the blocking antibodies in the albumin test We found a zone I suspected that we had another variety of blocking antibody and I almost committed myself except that when I did the adsorption experiment I found after treatment of the serum with Rh positive blood I did not remove this zone effect So far as my own findings are concerned I am a little bit less certain but I think as Dr Race pointed out the most striking observation is that on the serum which deteriorated and lost everything except your third order antibody

Dr Scudder I think it is excellent that you have gone on into electrophoresis studies and have found these antibodies present in the gamma globulin When I visited Dr Cohn s laboratory and spoke to Dr Diamond concerning such studies especially those concerning the sedimentation constants I found that such studies had not been completed as yet and I would like to ask Dr Hill whether he has carried on any of these types of examinations and in his electrophoresis studies whether this serum was kept in the frozen or in the liquid state

Dr Haberman In response to the question asked by Dr Scudder we can state that the electrophoresis tests were done at 0 5°C In one case the serum had been kept froz n before electrophoresis patterns were made The pattern shown was made from an unfrozen serum I b lieve that Dr Hill will have something to say concerning studies done on the antibodies by us In response to Dr Hattersley s question concerning the production of developing serum by using the purified gamma globulin as the antigenic stimulus theoretically it would appear that such a substance used as an antigen in rabbits would be the ideal one for the production of anti human globulin serum This of course rests on th assumption that all human antibodies are gamma globulin in nature If however gamma globulin do s not represent all of the human antibodies then this type of antiserum would fail to demonstrate some antibodies The

ACUTE RENAL INSUFFICIENCY DUE TO INCOMPATIBLE TRANSFUSION AND OTHER CAUSES, WITH PARTICULAR EMPHASIS ON MANAGEMENT

By E E Muirhead M D, A E Haley M D, Sol Haberman Ph D and J M Hill M D

ACUTE renal insufficiency may result from a series of different causes including incompatible (hemolytic) blood transfusion prolonged hypotension, crush injury burns, sulfonamide and carbon tetrachloride intoxication [1] The renal lesions in these cases which have been demonstrated to be morphologically quite similar are often reversible The mortality rate in this group of patients however has been alarmingly high As an example one may cite Lucke s conclusion that once the cardinal signs of renal insufficiency (oliguria excretion of heme pigment azotemia and hypertension) appear the mortality rate is approximately 90 per cent

The poor past record in the management of these cases may be attributed to one of two general causes (1) the injury to the body is so great as to preclude the success of any type of management or (2) the type of management in the past has not been conducive to the proper recovery of these cases Experience with such cases makes us agree with the latter view It is proposed to present evidence supporting such a stand

INCOMPATIBLE BLOOD TRANSFUSIONS

Statistics of the past from various sources have indicated a prominent rate of incompatible (hemolytic) transfusion reactions Kilduffe and DeBakey [3] compiled the results of 43 284 blood transfusions by 18 different groups of workers There were 1 8 hemolytic reactions in every 1000 transfusions and of these 1 04 per 1000 (57 7 per cent) were fatal

Approximately three fourths of the above reports appeared prior to the discovery of the Rh antigens The routine use of Rh typing in transfusions no doubt would have lowered the quoted hemolytic reaction rate Yet it may well be that isoimmunization by various CDE cde (Rh Hr) sub group antigens and other less understood blood antigens plus regrettable clerical technical errors will continue to make this an important problem Certainly the present upsurge in the use of whole blood transfusions lends additional emphasis to this question (fig 1)

Clinical Course of Hemolytic Transfusion Cases The clinical course of a patient experiencing a hemolytic reaction may be divided into three phases as presented in table 1

Certain features of these phases are considered to be of diagnostic and therapeutic value The *first phase* may be termed the stage of reaction shock The onset of the reaction is usually sudden and the patient displays the following manifestations apprehension generalized tingling sensations of the skin tightness in

From the William Buchanan Blood Center Baylor Hospital Dallas Texas

at fairly frequent intervals may result in some success in raising agglutinin titres to usable levels More impressive however is the fact that some individuals in whom we have undertaken further immunization by doses of Rh positive cells have shown only pure saline agglutinins even after many doses and many blood collections In one case in particular the titre continued to rise with no evidence of blocking or third order antibodies until a titre in excess of 100 000 was reached On the other hand we have also seen cases where an almost pure third order type of antibody was observed throughout the entire course of immunization with only transient very low levels of saline agglutinin or blocking antibody being present

I should like to thank Dr Race and his coworkers for providing us with this fine tool for the study of antibodies namely their anti human globulin method which for convenience we have termed the developing test

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the chest severe backache dyspnea, cyanosis and mental confusion A chill fever response and hypotension are quite common The duration of the hypotension varies and may be influenced by other coexisting factors (blood loss anesthetic agent anemia operation, etc) Prolonged hypotension may in itself be conducive to factors that cause renal damage [4 5] A hemorrhagic tendency is not unusual soon after the reaction

The patient may receive 40 to 500 cc of the incompatible blood before the above manifestations become severe The factors that determine the necessary volume of infused blood for the evident reaction to appear have not been fully elucidated The titer of the implicated antibody in the recipient and the type of antibody (agglutinating blocking and/or cryptagglutinoid types[6]) are concerned with this aspect of the pathogenesis of the reaction According to the observations of Hill Haberman and Jones[7] the violence and degree of hemolysis may also be related to the number of antibody antigen combinations (multiple R B C antigens and/or antigens determined by single or double gene doses) This concept indicates that homozygous red cells and red cells with multiple antigens (as CDE/cde, etc) for which antibodies are available react more promptly and more violently The antibody titers may actually be diminished during this phase due to their adsorption by the specific antigen or antigens

TABLE 1 —*Clinical Course Hemolytic Reaction Case*

I Reaction Shock (1st day)	II Renal Insufficiency (1 12 days)	III Salt Losing Diuresis (8th 16th day)
Hemolysis and hypotension	Tubular damage	Tubular recovery or regeneration
1 Sudden onset	1 Oliguria heme casts	1 Copious diuresis
2 Apprehension backache etc	2 Azotemia and hypertension	2 Severe dehydration if water and salt not supplied
3 Dyspnea cyanosis	3 Elevated serum K	3 Recovery
4 Hypotension mental confusion	4 Depressed serum Na Cl CO_2 CP Ca	
5 Chill fever	5 Rising titers of agglutinins and cryptagglutinoids	
6 Hemoglobinemia hemoglobinuria		

Concerning the hemolysis it should be mentioned that in an average sized individual it requires some 40–60 cc of hemolysed blood to liberate a sufficient quantity of hemoglobin into the circulating plasma in order to give rise to the appearance of hemoglobin in the urine The studies of Yuile[8] have placed the renal threshold in animals for free hemoglobin in the vicinity of 80–150 mg per 100 cc plasma

The *second phase* is the phase of renal insufficiency By far the most common cause of death has been uremia and associated complications Oliguria is marked but usually is not static and each day a slightly greater volume of urine is excreted In our experience complete anuria has not been noted Associated with the oliguria there is a marked decrease in the excretion of solid matter by the kidneys Not only is the solid output scanty but the concentration of various ingredients is substan-

tially lowered For instance the urine urea concentration may not exceed 5–20 per cent of normal

The urine contains pathologic ingredients during the first few days Free hemoglobin gives the first few specimens a dark red or reddish brown color Hemoglobinemia and its attendant hemoglobinuria usually disappear within 24 to 36 hours Proteinuria is definite however, even after the hemoglobinuria subsides It amounts at first to 500–1000 mg per 100 cc of urine, and gradually decreased to 50–100 mg per cent within 4 to 5 days The presence of casts containing the breakdown products of hemoglobin (heme casts) is an important diagnostic feature The heme may give a positive benzidine reaction This material may cause a brownish or brownish green sediment in the urine which gradually subsides during the early days The presence of intact red blood cells in the urine is common and white blood cells frequently in clumps are present during the oliguric period The urine specific gravity is depressed (1 005–1 010) and remains low for a varying period after recovery

A mounting azotemia and slight to moderate hypertension occur in conjunction with the above changes

The electrolyte pattern of the plasma (or serum) and blood is altered Early the serum potassium concentration is elevated a change apparently related to the breakdown of red cells rich in potassium Other common changes include a decrease in the concentration of serum sodium blood (or plasma) chlorides and the carbon dioxide combining power of the plasma The mechanisms involved in the hyponatremia and hypochloremia are not clear at present The degree of hyponatremia frequently exceeds the hypochloremia The serum calcium concentration is likewise frequently depressed The extent of these deviations naturally is influenced by the type of management and the severity of the renal insufficiency

During the stage of renal insufficiency the antibody titer of the patient s serum may become elevated a very specific evidence of the occurrence of hemolysis It is important to observe the titer of various types of antibodies agglutinating blocking [9 10] and albumin[11] or cryptagglutinoid[1] types

The renal insufficiency phase lasts 8–12 days apparently depending a great deal on the management and culminates either in a fatality in uremia and other complications or in recovery following a copious diuresis (third or salt losing diuresis phase) The diuresis becomes marked following a gradual daily increment in the 24 hour urinary output (as in Case 1 12 cc 30 74 39 100 124 206 540 1480 2300 6670 cc etc) The ability of the kidneys to concentrate waste products improves and these two factors increase the solid output This gradual increase in both urinary volume and solid output strongly suggests a gradual recovery of damaged renal elements

During the early phases of the diuresis not only is the 24 hour urine urea output markedly elevated but at the same time the 24 hour urine salt output (mainly NaCl) may be greatly increased Some 20 to 40 grams of salt may be lost daily by this mechanism This finding indicates that the renal tubules have recovered sufficiently to allow for urine volume production but that the recovery is as yet insufficiently advanced to allow for the conservation of water and salts

Such failure to conserve water and salts lasts a few days only unless other complications exist (i e another superimposed renal disease) Our observations indicate that the kidneys at this stage will discard body water and salts even to the extent of causing pronounced dehydration or even death Within certain limits such diuresis occurs whether or not water from sources outside the body is furnished This feature simulates the cases designated by Thorn[13] as salt losing nephritis

The therapeutic implications of the third or diuresis phase are evident It is essential to replace water and salts lost from the body The latter may be accomplished by mouth or by vein depending on the patient s condition As soon as possible the patient should be allowed to adjust his own intake and output

Renal Lesions Excluding sudden fatality at the onset of the reaction, apparently a rare occurrence, renal complications are the main cause of deleterious sequelae The combination of factors necessary for the production of the major damages to the kidneys are not fully understood at present One cannot state that hemolysis alone and the intravascular liberation of products of damaged red cells are all that are necessary Experimentally an animal s venous hemoglobin concentration may be rapidly depressed to low levels as 25 per cent of normal through the medium of intravascular hemolysis (injection of anti dog red cells rabbit serum into dogs) without the development of evident renal insufficiency [14–16] Clinically various hemoglobinemias with hemoglobinuria have been repeatedly observed without the development of renal insufficiency Substantial quantities of hemoglobin solution have been infused intravenously into humans without subsequent renal insufficiency [8] [17] Yuile[18] has shed considerable light on this question by demonstrating experimentally the consistent development of renal insufficiency in animals following *intravenously administered hemolysed red cells after the kidneys* had been damaged by either the temporary constriction of renal vessels or by the administration of a nephrotoxic substance as sodium tartrate These findings imply that primary renal damage as might occur clinically in prolonged hypotension severe anemia acute hypovolemia is a prerequisite which together with hemolysis gives rise to the more severe renal damage and renal insufficiency Other etiologic explanations have been reviewed by Lucke

The role of the urinary pH at the time of the hemolysis has been the source of much controversy [19– 5] There is good reason to doubt any benefit of an excessive alkali intake once the renal damage has occurred Indeed a sodium overload at this time may be distinctly dangerous

Ross [5] has reviewed the subject and summarized the various factors involved in the renal damage by singling out the following lowered blood pressure, vasoconstriction of renal vessels reduced renal blood flow lowered alkali reserve and the secretion of large amounts of hemoglobin or hemoglobin derivatives in an acid urine

The morphologic alterations of the kidneys have received much attention [1] [27– 9] Such terms as hemoglobinuric nephrosis and lower nephron nephrosis have been applied to these lesions The kidneys are usually enlarged and may weigh one and one half to two times normal The cortex is pale the medulla is dark dusky and presents a well striated appearance Bulging of the cut surface

denotes an increased subcapsular tension Microscopically the main damage involves the lower segment of the nephron a feature greatly stressed by Lucke and others Here degeneration or necrosis of the tubular epithelium are evident in a segmental distribution Where necrosis occurs foci of inflammation can be seen (accumulation of neutrophils lymphocytes eosinophils and macrophages) Thrombosis of thin walled veins may occur in the vicinity Heme casts may be prominent in the form of brownish granular or eosinophilic granular material in distal segments and collecting tubules and interstitial edema is usually evident Peritubular hyperemia is particularly striking in the medullary region The upper segments are the seat of acute parenchymatous degeneration at times fatty degeneration Leak-

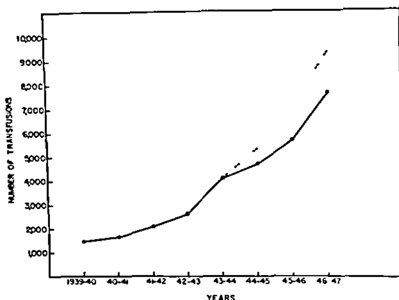


FIG 1 GROWTH OF THE BLOOD TRANSFUSION SERVICE OF THE WILLIAM BUCHANAN CENTER IN EIGHT YEARS

The solid line represents all transfusions given at Baylor Hospital the dotted line represents the total transfusions given both within and outside Baylor Hospital

age of plasma proteins through the glomerular membrane is evident particularly early Otherwise morphologically the glomeruli appear undisturbed

Regeneration of the distal segment becomes evident by the eighth to tenth day The damaged lining cells slough into the lumen and are replaced by new cells The young cells are flat at first but later gain more and more substance The inflammatory foci become replaced by fibrous tissue

Review of Cases The present report is presented in two parts the first is concerned with 18 cases of hemolytic transfusion reactions and the second part deals with 10 cases of acute renal insufficiency (or lower nephron nephrosis) due to o her causes

The growth of the blood transfusion service of the William Buchanan Blood Center of Baylor Hospital is indicated in Figure 1 It is believed that this curve is indicative of the general trend in blood transfusion services throughout the country and emphasizes the importance of the problem under consideration During these

Such failure to conserve water and salts lasts a few days only unless other complications exist (i e another superimposed renal disease) Our observations indicate that the kidneys at this stage will discard body water and salts even to the extent of causing pronounced dehydration or even death Within certain limits such diuresis occurs whether or not water from sources outside the body is furnished This feature simulates the cases designated by Thorn[13] as salt losing nephritis

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later (ninth, tenth, and sixteenth day respectively) the diuresis likewise was late in its onset Such late onset of diuresis may be related to the management of the case

REPORT OF CASES

Case 1 A white female aged 23 years delivered an icteric but otherwise apparently normal baby Subsequently the baby s red blood cells yielded a positive Coomb s (developing) test [12, 30] an indication of an attack on the infant s red cells by maternal antibodies (erythroblastosis fetalis) A prior pregnancy had terminated with the delivery of a still born infant at seven months

Following delivery an adherent placenta was extracted manually during ether anesthesia Profuse bleeding occurred and the systolic blood pressure dropped to 80 mm Hg At this time the patient was given a transfusion of blood her husband acting as the donor (The wife and husband had been typed as Rh positive elsewhere) After 380 cc of blood had been taken there was a chill mental dullness and one hour later the oral temperature was 103 F Chilly sensations lasted 1½ hours and during this period a lethargic state existed The blood pressure was 150/90 pulse rate 146 Bleeding from the uterus continued despite packing and during the following 14 hours the arterial blood pressure gradually dropped to an imperceptible level The blood pressure remained very low for at least one hour and the patient was described as cadaveric in appearance The patient had been given 1500 cc of dextrose in normal saline by vein

TABLE 2.—*Studies Related to Case 1*

	Anti C	Anti-c	Anti D	Anti E	Anti e	Estimated Genotype*
Mrs A	−	+	−	−	+	cde/cde
Mr A	+	+	+	+	+	CDe/cDE
Infant A	−	+	+	+	+	cDE/cde

During the above mentioned 14 hours blood samples were sent to the William Buchanan Center It was determined that the patient was Rh negative i e type cde/cde* and the husband who had given the blood was D* positive (Rh positive) and his estimated genotype[31] was designated as CDe/cDE * When the infant was tested and found to be type cDE/cde * the father s estimated type was proved correct (See Table 2)

As soon as possible the patient was given 1000 cc of cde/cde blood † Since bleeding continued and the B P remained at 80 mm Hg 2500 cc of iso-osmotic citrated plasma were given during the ensuing four hours and this was followed by 600 cc of cde/cde blood The systolic BP was 120 mm Hg Acute pulmonary edema developed and 300 cc of blood were withdrawn by vein The patient was then transported 80 miles via plane to Baylor Hospital On arrival the BP was 120/100 pulse rate 150 and respiratory rate 60 Diffuse pulmonary edema existed and 500 cc of blood were immediately withdrawn from the right femoral artery Prompt improvement resulted The BP became 112/78 pulse rate 104 and respirate rate 26

Oozing of blood from the uterus continued for 36 hours but contraction of the uterus progressed satisfactorily This postreaction hemorrhagic tendency was likewise noted in relation to all skin punctures The blood clotting time (Lee White) was 8 minutes

Since overloading in the intravascular compartment had occurred no fluids were administered by any route for 48 hours After this period 1000 to 1500 cc of fluid were given daily as a high caloric formula No vomiting occurred and the formula was well tolerated

* Fisher Race terminology[31, 32] Cf paper by Race this issue

† Since a theoretical possibility of E immunization existed it became necessary to make certain the donor blood was E negative by the use of anti E serum

8 years, 28 630 blood transfusions were given in this hospital and there were 17 known hemolytic reactions or an incidence of 0 593 reactions per 1000 transfusions Of these reactions 3 (17 6 per cent) were fatal An additional case brought to Baylor Hospital for treatment brings the total to 18

The hemolysis was related to the ABO antigens in 4 cases and to the Rh (CDE) antigens in 10 cases In 4 instances, the exact cause of the hemolysis could not be determined

In all cases a combination of well-defined manifestations established the existence of this type of reaction Included were the following the nature of the onset of the reaction with its violent aspects failure of elevation of the red cell count or hemoblogin concentration hemoglobinuria (at times hemoglobinemia was demonstrated) oliguria and renal insufficiency in certain cases an elevated serum bilirubin or icterus index level the development of the proper antibodies and the *establishment of the incompatibility by rechecks of the donor s and recipient s* blood

TABLE 1 A —*Volume of Blood Given Before Onset of Reaction*

Volume blood	Frequency Cases
cc	
40– 100	6
101– 200	3
201– 300	2
301– 400	3
401– 500	2
501–1000	2
Total	18

The volume of blood administered before the reaction became definite varied between 40 cc and 1000 cc The average volume for this purpose was 281 cc The frequency distribution of the volume for all 18 cases is given in Table 1–A

All cases displayed a chill and fever at the onset of the reaction The chill lasted between 8 and 45 minutes and the temperature elevations averaged 3 7°F (range 2°–6°) within one to two hours after the onset of the reaction Over 50 per cent of the cases displayed the following manifestations in the early phases of the reaction apprehension generalized tingling sensations or muscular cramps severe backache, cyanosis mental cloudiness and hypotension Unless complicated (blood loss etc) the hypotension was evanescent lasting 10–15 minutes Twelve cases (66 6 per cent) developed hemoglobinuria which was demonstrable in the first or second urine specimen Ten cases (55 5 per cent) subsequently developed oliguria and azotemia The azotemia was always definite within 24 hours (blood urea 80–100 mg per 100 cc) and mounted each day until an adequate urinary output appeared or the case terminated fatally The average time for the azotemia to reach its peak was 8 days, following which there was either the onset of diuresis or fatal deterioration It follows that in the 3 non fatal cases in which the peak of azotemia developed

The diuresis began on the eleventh day It is of interest that the patient was not seen other than by the nursing staff for approximately 10 hours during the night of the eleventh day Between the time the patient was seen in the evening by one of us and the following morning the profuse phase of diuresis commenced No additional fluids had been received during this time During this short period the patient displayed definite signs of dehydration (extracellular water salt loss) dry tongue and mouth ropey saliva parchy lips soft eyeballs dry skin Certain mental aberrations were noticed marked decrease in psychomotor activity blocking phenomena a lack of interest in her baby ideas of reference concerning members of the staff auditory hallucinations and delusions Following the replacement of water and salt these mental features disappeared with phenomenal rapidity

The following three days were crucial with regard to the urinary water and salt output The urinary volume amounted to 6 670 cc 8 770 cc and 6 840 cc respectively In these volumes the following corresponding quantities of salts were measured chlorides as NaCl 28 7 grams 35 2 grams 28 2 grams sodium

TABLE 3 —*Case 1*

Day	Blood Urea N	Blood Urea	CO_2 CP	Blood Cl	Serum Na	Serum K	Plasma Prot	Hg B
	mg /100 cc	mg /100 cc	V %	mg /100 cc	mEq/L	mEq/L	G/100 cc	G/100 cc
2	50	107	49		144	7 2		7 0
4	95	200	59	500	146	6 4	5 6	7 6
8	222	475	46	450	130	5 9		8 8
11–12	214	458	54	400	132	5 0	5 5	9 15
15–16	39 9	85 3	56	477	142	4 3	6 9	
17–18	30	64	64	480	145	4 4		
19	25	53	68	490				

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine Na	Total Urine K	BP
	cc	cc	gm	gm	gm	gm	mHg
1– 2	4 800	12	0 048	0 029			
3– 4	180	104	0 278	0 342			112/78
5– 8	3 000	469	2 319	1 949			
9–12	5 155	10 990	68 33	44 54	25 53	3 29	135/94
13–16	25 715	27 985	122 9	93 1			128/88
17–18	6 600	10 345	40 5	15 6			

16 68 grams 19 4 grams and 19 35 grams potassium 2 35 grams 2 2 grams 1 85 grams At the same time the 24 hour urine urea output amounted to 35 9 grams 44 grams and 35 3 grams (fig 2)

At the time of the profuse diuresis the water salt needs were judged in accordance with the measured output in the urine which meant that we were always several hours behind the actual replacement needs of the patient For each 24 hours during the above mentioned three day period the patient received by vein 6000 cc of fluid 5000 cc of which contained the following bulk quantities of salt 32 grams of NaCl 12 5 grams of $NaHCO_3$ and 0 9 grams of KCl

After the three day period (fourteenth day) the urine urea salt and water output began receding The intravenous fluids were discontinued The patient was placed on a general diet and allowed to regulate her own fluid intake The 24 hour salt output dropped to 6–10 grams and the urea output dropped to near 20 grams The daily urine volume remained high (3000–6000 cc)

Concomitantly with the diuresis the blood urea concentration dropped precipitously to normal levels The blood chlorides serum sodium concentrations and CO_2 combining power of plasma were elevated back to normal On the seventeenth day despite these reversals the urea clearance was 20 per cent of normal the P S P test 32 per cent of normal (first hour 12 5 per cent second hour 19 5 per cent) The urine specific gravity fluctuated between 1 006 and 1 011 from the second day until discharge

Water soluble vitamins (B and C) were given parenterally. An average of 4 grams of sodium bicarbonate were given daily. On one occasion when the serum Na concentration was lowered to 132 mEq/L 14 grams of sodium bicarbonate were given per rectum.

The course of this case can be traced in Figure 2 and Table 3. On the first day the plasma hemoglobin concentration (modified Bing and Baker method[34]) was 512 mg. per cent dropping to 244 mg. per cent by the second day and disappearing by the third day. Methemalbumin[35] was detected in the plasma samples of the first day. During the first nine days a marked oliguria existed but this gradually lessened (succeeding daily urine volume 12, 30, 74, 39, 100, 124, 206, 540 and 1480 cc.). The first two urine specimens contained hemoglobin in solution. For four days the urine specimens contained brownish granular casts (heme casts). At all times the urine specific gravity varied between 1.008–1.010. The blood pressure

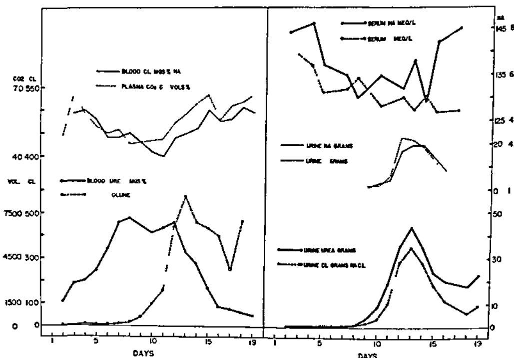


Fig. 2. Laboratory Findings during the Three-phase Management as Applied to Case 1 of the Hemolytic Transfusion Reaction Group

The patient received 380 cc. of incompatible blood. Notice the reciprocal relation of oliguria and azotemia, the gradual lowering of blood Cl, plasma CO_2 CP, serum Na and K during the oliguric period. The prominent output of Na, K, Cl and urea during the diuresis is well demonstrated. All of the variation changes develop through gradual transitions.

remained in the vicinity of 135/94 mm. Hg. The blood urea concentration was elevated from 110 mg. per cent on the second day to 458 mg. per cent on the eighth day. It can be noted that a gradual decline in blood chloride, serum Na and K concentration and the CO combining power of plasma took place during these eight days.

Antibodies against the C and D antigens appeared in the patient's serum. On the seventh day the titer for the agglutinating antibody was 1:4 and for the "cryptagglutinoid" type was 1:512.

The peripheral hemoglobin concentration was lowered from 8.55 grams per cent on the second day to 4.0 grams per cent on the fourth day. This hemodilution occurred during the "no-fluid intake" period. Three transfusions of concentrated red blood cells were given (750 cc. red blood cells plus about 200 cc. 5 per cent dextrose in distilled water). The hemoglobin concentration stabilized around 9 grams per cent. These transfusions plus penicillin therapy (30,000 units every 6 hours intramuscularly) constituted the only additional therapeutic measures during the oliguric period of eleven days.

The diuresis began on the eleventh day It is of interest that the patient was not seen other than by the nursing staff for approximately 10 hours during the night of the eleventh day Between the time the patient was seen in the evening by one of us and the following morning the profuse phase of diuresis commenced No additional fluids had been received during this time During this short period the patient displayed definite signs of dehydration (extracellular water salt loss) dry tongue and mouth ropey saliva parchy lips soft eyeballs dry skin Certain mental aberrations were noticed marked decrease in psychomotor activity blocking phenomena a lack of interest in her baby ideas of reference concerning members of the staff auditory hallucinations and delusions Following the replacement of water and salt these mental features disappeared with phenomenal rapidity

The following three days were crucial with regard to the urinary water and salt output The urinary volume amounted to 6 6-0 cc 8 770 cc and 6 840 cc respectively In these volumes the following corresponding quantities of salts were measured chlorides as NaCl 28 7 grams 35 2 grams 28 2 grams sodium

TABLE 3 —*Case 1*

Day	Blood Urea N	Blood Urea	CO_2 CP	Blood Cl	Serum Na	Serum K	Plasma Prot	Hg B
	mg /100 cc		*V %*	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*	*G/100 cc*
2	50	107	49		144	7 2		7 0
4	95	200	59	500	146	6 4	5 6	7 6
8	222	475	46	450	130	5 9		8 8
11–12	214	458	54	400	132	5 0	5 5	9 15
15–16	39 9	85 3	56	477	142	4 3	6 9	
17–18	30	64	64	480	145	4 4		
19	25	53	68	490				

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine Na	Total Urine K	BP
	cc	*cc*	*gm*	*gm*	*gm*	*gm*	*mHg*
1– 2	4 800	12	0 048	0 029			
3– 4	180	104	0 278	0 342			112/78
5– 8	3 000	469	2 319	1 949			
9–12	5 155	10 990	68 33	44 54	25 53	3 29	135/94
13–16	25 715	27 985	122 9	93 1			128/88
17–18	6 600	10 345	40 5	15 6			

16 68 grams 19 4 grams and 19 35 grams potassium 2 35 grams 2 2 grams 1 85 grams At the same time the 24 hour urine urea output amounted to 35 9 grams 44 grams and 35 3 grams (fig 2)

At the time of the profuse diuresis the water salt needs were judged in accordance with the measured output in the urine which meant that we were always several hours behind the actual replacement needs of the patient For each 24 hours during the above mentioned three day period the patient received by vein 6000 cc of fluid 5000 cc of which contained the following bulk quantities of salt 32 grams of NaCl 12 5 grams of $NaHCO_3$ and 0 9 grams of KCl

After the three day period (fourteenth day) the urine urea salt and water output began receding The intravenous fluids were discontinued The patient was placed on a general diet and allowed to regulate her own fluid intake The 24 hour salt output dropped to 6–10 grams and the urea output dropped to near 20 grams The daily urine volume remained high (3000–6000 cc)

Concomitantly with the diuresis the blood urea concentration dropped precipitously to normal levels The blood chlorides serum sodium concentrations and CO_2 combining power of plasma were elevated back to normal On the seventeenth day despite these reversals the urea clearance was 20 per cent of normal the P S P test 32 per cent of normal (first hour 12 5 per cent second hour 19 5 per cent) The urine specific gravity fluctuated between 1 006 and 1 011 from the second day until discharge

Water soluble vitamins (B and C) were given parenterally An average of 4 grams of sodium bicarbonate were given daily On one occasion when the serum Na concentration was lowered to 132 mEq/L 14 grams of sodium bicarbonate were given per rectum

The course of this case can be traced in Figure 2 and Table 3 On the first day the plasma hemoglobin concentration (modified Bing and Baker method[34]) was 512 mg per cent dropping to 244 mg per cent by the second day and disappearing by the third day Methemalbumin[35] was detected in the plasma samples of the first day During the first nine days a marked oliguria existed but this gradually lessened (succeeding daily urine volume 12 30 74 39 100 124 206 540 and 14?0 cc) The first two urine specimens contained hemoglobin in solution For four days the urine specimens contained brownish granular casts (heme casts) At all times the urine specific gravity varied between 1 008–1 010 The blood pressure

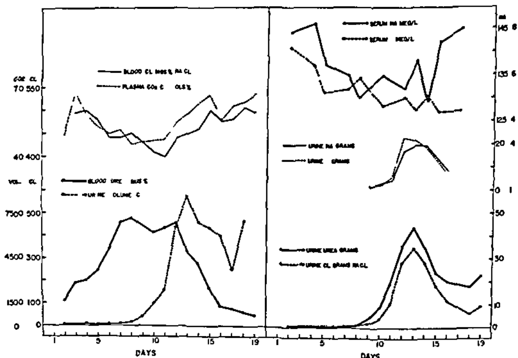


Fig 2 Laboratory Findings during the Three phase Management as Applied to Case 1 of the Hemolytic Transfusion Reaction Group

The patient received 380 cc of incompatible blood Notice the reciprocal relation of oliguria and azotemia the gradual lowering of blood Cl plasma CO_2 CP serum Na and K during the oliguric period The prominent output of Na K Cl and urea during the diuresis is well demonstrated All of the variation changes develop through gradual transitions

remained in the vicinity of 135/94 mm Hg The blood urea concentration was elevated from 110 mg per cent on the second day to 458 mg per cent on the eighth day It can be noted that a gradual decline in blood chloride serum Na and K concentration and the CO_2 combining power of plasma took place during these eight days

Antibodies against the C and D antigens appeared in the patient s serum On the seventh day the titer for the agglutinating antibody was 1 4 and for the cryptagglutinoid type was 1 512

The peripheral hemoglobin concentration was lowered from 8 55 grams per cent on the second day to 4 0 grams per cent on the fourth day This hemodilution occurred during the no-fluid intake period Three transfusions of concentrated red blood cells were given (−50 cc red blood cells plus about 200 cc 5 per cent dextrose in distilled water The hemoglobin concentration stabilized around 9 grams per cent These transfusions plus penicillin therapy (30 000 units every 6 hours intramuscularly) constituted the only additional therapeutic measures during the oliguric period of eleven days

TABLE 4—*Case 2*

Day	Blood Urea N	Blood Urea	CO_2 CP	Blood Cl	Serum Na	Serum K	Hg B
	mg 100		V %	mg/100 cc	mEq L	mEq L	G/100 cc
2	27	58	45				13 4
4	58	123	60	400	140	6 2	9 4
6	71	153	64	360	143	4 5	
8	57	123	49	360			10 1
10	69	145	59	490			9 75
13	37	80	62	510			
16	24	51	52	510	141	5 2	

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine Na	Total Urine K	BP
	cc	cc	gm	gm	gm	gm	mHg
1- 2	3 730	405	1 37	0 98	0 33	0 063	120/82
3- 4	10 280	2 095	5 4	5 0	1 644	0 49	124/96
5- 8	18 230	17 035	44 0	-5 7			142/100
9-12	16 700	17 310	88 6	26 9			158/96
13-15	13 500	11 460	66 7	24 7			160/100

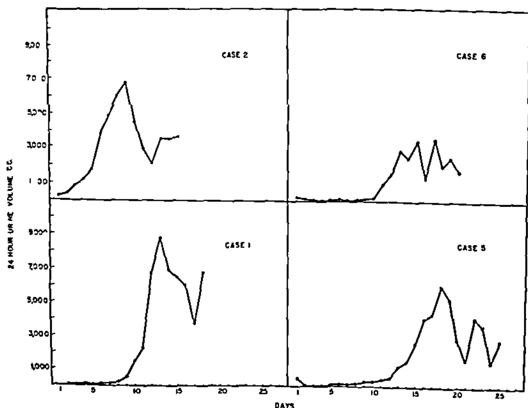


FIG 3 THE OLIGURIA DIURESIS CURVES OF FOUR CASES OF THE HEMOLYTIC REACTION GROUP ARE DEPICTED THE CORRESPONDING CASE NUMBERS ARE GIVEN

The patient was dismissed ambulant on the nineteenth day Three months later the urea clearance was 74 per cent of normal

Comment This case was a very severe one Aside from the hemolytic reaction other distinct factors conducive to renal damage existed namely hemorrhage with prolonged hypotension severe anemia uterine damage due to removal of an adherent placenta The management based as it was on the outstanding features of the three clinical phases, may be designated as the three phase management

In the first phase treatment of the blood loss and hypotension became vital An understanding of the various Rh antigens made it possible to administer 1600 cc of blood with confidence Plasma infusions were given for the oliguria until more blood could be made available A plasma overload resulted from a misunderstanding but it yielded rapidly to blood withdrawal A close check on venous distension respiratory embarrassment and the presence of crepitant rales at the bases of the lungs can prevent such complications readily

Since blood loss continued there was no hesitancy in giving concentrated red cell transfusions for the subsequent severe anemia

During the phase of renal insufficiency no attempt was made to force the kidneys into action Conversely water was given only to substitute for the insensible loss and the scanty urinary output Time was allowed to pass in order to allow the kidneys opportunity to regenerate Until such time the nitrogenous waste products were allowed to accumulate

On the above regime diuresis was spontaneous on the twelfth day The water salt deficit was replenished The remarkable recovery is considered more than coincidental in view of the severity of this case

Case 2 A white female aged 20 years had pyelitis with severe chills and fever at the time of delivery of normal twins Urine cultures yielded a growth of Pseudomonas aeruginosa

The patient was of Typ O D positive Sixteen hours following the delivery through an error she received 100 cc of Type A D positive blood The series of manifestations included abdominal rigidity back pain cramping of legs nausea pressure on head cyanosis weak pulse and a chill lasting 35 minutes Within two hours the rectal temperature was 105 8 F During the subsequent 12 hours however the temperature was subnormal (92 4 –94 F) A urine specimen five hours after the reaction contained much brownish granular debris (hemoglobinuria) and heme casts Additional urine specimens were red in color

The chills and fever of pyelitis continued during the next four days (daily temperature 102 –103 F) Profuse diaphoresis occurred during this period requiring repeated changes in bed linen due to wetness Streptomycin therapy was initiated on the third day following the reaction and was continued for six *days thereafter (0 25 gram every 3 hours intramuscularly for four days then 0 125 gram per dose)* During this time the temperature dropped to normal levels and remained so

Table 4 contains data on this case In addition to the hemoglobinuria heme casts marked proteinuria and low specific gravity of urine the patient developed oliguria azotemia hypertension and hypochloremia The CO_2 combining power of plasma and serum sodium concentration were maintained within normal bounds by the administration of sodium bicarbonate For four days the urine urea output was low (total 6 77 grams) During these four days the intake of fluids (14 010 cc) far exceeded the urinary output (2 500 cc) but profuse sweating was constant and development of subcutaneous edema was not evident No pulmonary edema was detected The fluids given were salt free with the exception of three units of plasma and 500 cc of blood on the first day

Diuresis began on the sixth day and reached its peak on the eighth day (fig 3 Case 2) There was a fairly rapid recession of all abnormalities except the hypertension which persisted up to the time of dis-

TABLE 4—*Case 2*

Day	Blood Urea N	Blood Urea	CO CP	Blood Cl	Serum Na	Serum K	Hg B
	mg /100 cc		*V %*	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*
2	27	58	45				13 4
4	58	123	60	400	140	6 2	9 4
6	71	153	64	360	143	4 5	
8	57	123	49	360			10 1
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13	37	80	62	510			
16	24	51	52	510	141	5 2	

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine Na	Total Urine K	BP
	cc	*cc*	*gm*	*gm*	*gm*	*gm*	*mHg*
1- 2	3 730	405	1 37	0 98	0 33	0 063	120/82
3- 4	10 280	2 095	5 4	5 0	1 644	0 49	124/96
5- 8	18 230	17 035	44 0	25 7			142/100
9-12	16 700	17 310	88 6	26 9			158/96
13-15	13 500	11 460	66 7	24 7			160/100

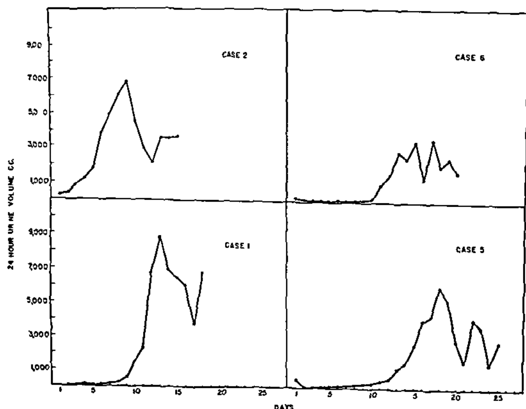


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	mg /100 cc	*mg /100 cc*	[illegible]	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*
2	27	58	45				13 4
4	58	123	60	400	140	6 2	9 4
6	71	153	64	360	143	4 5	
8	57	123	49	360			10 1
10	69	145	59	490			9 75
13	37	80	62	510			
16	24	51	52	510	141	5 2	

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine Na	Total Urine K	BP
	cc	*cc*	*gm*	*gm*	*gm*	*gm*	*mHg*
1- 2	3 730	405	1 37	0 98	0 33	0 063	120/82
3- 4	10 280	2 095	5 4	5 0	1 644	0 49	124/96
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9-12	16 700	17 310	88 6	26 9			158/96
13-15	13 500	11 460	66 7	24 7			160/100

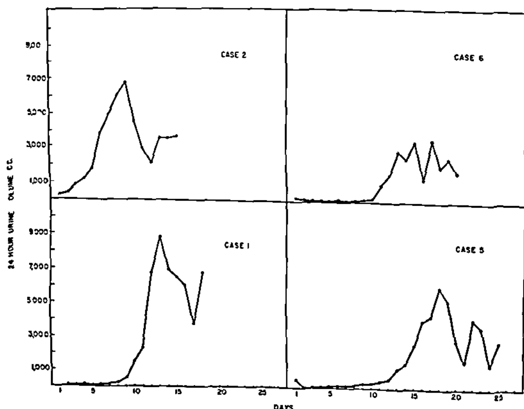


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charge sixteen days after the reaction The urine specific gravity fluctuated between 1 003 and 1 013 from the second day until the day of discharge

Comment Pyelitis of pregnancy existed prior to the incompatible transfusion but was not attended by azotemia as indicated by a control blood urea N level of 14 mg per 100 cc Azotemia and renal insufficiency however were definite following the reaction

The treatment in this case was of the same three phase type as mentioned in the discussion of Case 1 During the first phase the patient was given 500 cc of

TABLE 5 —*Case 3*

Day	Blood		CO CP	Blood Cl	Serum Na	Serum K	Plasma Prot	Hg B
	Urea N	Urea						
	mg /100 c		1 c	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*	*G/100 cc*
2	63	133		510			5 7	10 8
7–8	107	229	58	400				9 15
12	115	247	48	380				10 4
16	150	310	49	360				
19	83	178	31	620	150	4 84		10 8
22	56	120	30	420				11 4
26	43	92	56	440				10 4
29	37	80	54	400				
35	20	43		430	142	4 1	6 6	15 0
46	15	32	58	480			7 1	

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Perit Urea
		cc	*gm*	*gm*	
1– 4	5 500	325			
5– 8	7 600	575			
9–12	6 000	1 935			
13–16	6 650	6 600			
17–19	6 235	6 155	29 6	18 61	46 0
20–22	8 440	8 600	36 4	11 1	30 85
23–28	25 670	20 565	156 7	35+	
29–34	15 600	17 770	133 8	24 3	
35–40	19 710	16 585	120 3	35 2	
41–46	17 600	21 820	103 8	42 5	

compatible blood During the second phase the management consisted mainly of replacement of water loss via sweat and urine and a salt poor diet The CO_2 combining power of plasma was maintained within normal limits by an alkalinizing mixture (citrate carbonate) During the third phase the intake was made to replace the copious output The abatement of the fever of pyelitis was synchronous with the streptomycin therapy Mental clarity was present throughout and there was no vomiting One month later the following observations were made blood urea concentration 31 mg per 100 cc urea clearance 58 per cent of normal

Case 3 This case has been previously discussed[33] and will be touched on briefly only Peritoneal irrigation was conducted between the seventeenth and twenty second days (table 5)

The patient was of Type O D positive and received 175 cc of Type A_1 D positive blood The chain of events included nausea generalized tingling sensations hypotension for 20 minutes cyanosis chill fever mental confusion and hemoglobinuria Subsequent renal insufficiency with hyper tension developed

The azotemia reached its peak on the sixteenth day At this time the following complications existed gallop rhythm apical systolic murmur, pericardial friction rub generalized subcutaneous and pulmonary edema muscular twitchings

Peritoneal irrigation was conducted for 5 5 days During the first three days the irrigation was conducted with crystalloid solutions and the water salt overload was aggravated Diuresis on the nineteenth day coincided with the washing out of salt from the body via the peritoneum by means of 5 per cent dextrose in distilled water The urine specific gravity remained low (1 005–1 016) throughout

TABLE 6 —*Case 4*

Day	Blood		CO CO	Plasma Cl	Serum Na	Serum K	Plasma Prot	Hg B
	Urea N	Urea						
	mg /100 cc		V %	mg /100 cc	mEq/L	mEq/L	G/100 cc	G/100 cc
2	46	99						
4	100	214	62	510	153	3 0	6 5	9 5
8–9	81	174	22	580	142	4 2	4 5	5 6
11	57	123	26	600				8 15
12	68	145	43	520				8 55
14	86	183	50	500	134	4 9		
15	94	201	43	520				

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Perit Urea	Icterus Index
	cc	cc	gm	gm		
1– 2	2 350	290	1 9	1 1		82 8
3– 4	3 200	225	1 24	0 79		173 0
5– 9	6 800	1 155	3 24	2 26+	104 0+	100 0
10–11	4 300	595	1 26		11 0+	95
12	1 760	230	0 64	0 86		97 5
13–14	880	1 080	3 37	3 38		122 5
15	1 240	475				

Comment The necessary copious diuresis in this case was delayed to the nineteenth day It has been demonstrated that by this time regeneration of the renal tubular epithelium has usually taken place The delay in diuresis seemed related to the extracellular water salt overload A great deal of salt was cleared via the peritoneum by using a non salt containing irrigating solution

Continuous irrigation of the peritoneum with crystalloid solutions was complicated by a severe acidosis and water salt absorption

Case 4 This case has also been discussed elsewhere [3] and will be only briefly mentioned here Peritoneal irrigation was conducted between the third and eleventh days

The cause of the hemolysis was not determined in this case The recipient and all donors on repeated checks were found to be Type A_1 D positive Unmistakable acute hemolysis occurred A total of 2650 cc of blood were given over twelve days

Table 6 contains the essential features of the data Hemoglobinuria jaundice azotemia and hyper tension developed The peritoneal irrigation was responsible for the clearing of 115 grams of urea from

charge sixteen days after the reaction The urine specific gravity fluctuated between 1 003 and 1 013 from the second day until the day of di charge

Comment Pyelitis of pregnancy existed prior to the incompatible transfusion but was not attended by azotemia as indicated by a control blood urea N level of 14 mg per 100 cc Azotemia and renal insufficiency, however were definite following the reaction

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16	150	310	49	360				
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22	56	120	30	420				11 4
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17–19	6 235	6 155	29 6	18 61	46 0
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TABLE 6 —*Case 4*

Day	Blood		CO_2 CO	Plasma Cl	Serum Na	Serum K	Plasma Prot	Hg B
	Urea N	Urea						
	mg /100 cc		1 cc	mg /100 cc	mEq/L	mEq/L	G/100 cc	G/100 cc
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4	100	214	62	510	153	3 0	6 5	9 5
8–9	81	174	22	580	142	4 2	4 5	5 6
11	57	123	26	600				8 15
12	68	145	43	520				8 55
14	86	183	50	500	134	4 9		
15	94	201	43	520				

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Perit Urea	Icterus Index
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3– 4	3 200	225	1 24	0 79		173 0
5– 9	6 800	1 155	3 24	2 26+	104 0+	100 0
10–11	4 300	595	1 26		11 0+	95
12	1 760	230	0 64	0 86		97 5
13–14	880	1 080	3 37	3 38		1–2 5
15	1 240	475				

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the body At the same time however marked acidosis developed and there was evidence of water salt absorption The urine specific gravity remained low (1 006–1 012)

This patient s progress was unfavorable and she expired on the fifteenth day The autopsy findings demonstrated widespread pulmonary tuberculosis and the typical renal lesions of incompatible blood transfusion Renal tubular regeneration had advanced (Fig 8)

Comment This was an extremely complicated case The tuberculosis was extensive The anemia was of such magnitude as to demand transfusions Transfused red cells were promptly hemolysed by an undetermined mechanism Irrigation of the peritoneum for the renal insufficiency was followed by severe acidosis and evidences of water salt absorption

TABLE 7 —*Case 5*

Day	Blood		Blood Cl	Plasma Prot	Hg B
	Urea N	Urea			
	mg /100 cc		mg /100 cc	G/100 cc	G/100 cc
3	36	76			
4	40	85			
8	65	139	280		9 15
10	73	157		5 2	
11–12	93	200	340	6 5	
17	40	86			
21	63	133			8 55

Days	Intake	Urin Vol	BP
	c	c	mHg
2	4 360	50	110/60
3– 4	4 100	120	
5– 8	13 060	495	
9–12	10 925	1 460	124/100
13–16	4 690	9 125	
17–20	8 730	18 300	115/78
21–24	9 050	10 850	

Case 5 A white female 40 years of age had a hysterectomy following which there was vaginal bleeding and a lowered blood pressure (90/70 to 100/70 mm Hg) The patient received 2 000 cc of blood without reaction and was again operated on 36 hours later for the control of bleeding During the immediate postoperative period 2 000 cc of 5 per cent dextrose solution were given by vein At 16 hours post operatively a blood transfusion resulted in severe reaction after 250 cc had been given A severe chill lasted 8 minutes and the temperature was elevated to 103 4 F Nineteen hours later 100 cc of dark bloody urine were obtained by catheter Subsequently prominent azotemia hypochloremia and oliguria developed (table 7) There was no hypertension

For twelve days the intake far exceeded the urinary output The patient displayed generalized edema became irrational highly talkative loud and groaned frequently On the tenth day irregular muscular jerks of the arms and legs appeared and continued until the height of the diuresis on the seventeenth day Up until this time the fluid intake included the following by vein 18 000 cc of sixth molar sodium lactate solution 1 300 cc of 3 per cent NaCl solution 100 cc of concentrated plasma and 1000 cc of 1 8 per cent sodium sulfate solution

On the fifteenth day there occurred a grand mal convulsion The following day generalized rales over

the lung fields were noted Improvement began with the peak of the copious diuresis (seventeenth eighteenth and nineteenth days) From this time on recovery was rapid (Fig 3 Case 5) The patient was discharged walking 25 days after the reaction The urine specific gravity remained between 1 008 and 1 010 throughout

Comment During the first sixteen days the fluid intake far exceeded the urine output and even exceeded the urine volume plus the estimated insensible loss The intake of sodium was quite high and apparently eventuated the extracellular fluid *volume expansion On this regime mental signs and a convulsion occurred* The height of the diuresis was delayed to the eighteenth day

TABLE 8 —*Case 6*

Day	Blood		CO_2 CP	Blood Cl	Plasma Prot	Hg B
	Urea N	Urea				
	mg 100 cc		*% v*	*mg /100 cc*	*G /100 cc*	*G/100 cc*
2	50	110				8 55
6	85	180	38	340		16 1
8–9	90	190	52	350	5 5	16 4
13	106	227	50	380		15 0
16	45	100	68	360		14 45
19	20	43	72	420		13 85

Days	Intake	Urine Vol	BP
	cc	*cc*	*mHg*
1– 2	1 000	305	110/60
3– 6	3 150	175	
7– 9	2 600	155	
10–13	9 500	5 785	
14–16	1 600	7 425	
17–19	3 400	8 200	130/78

Case 6 A white female aged 30 years entered the hospital for metro-menorrhagia There had been one pregnancy in the past A severe hemolytic reaction occurred and was demonstrated to be due to the Rh (CDE) system of antigens The patient received 350 cc of blood in 45 minutes There were generalized tingling sensations nausea and vomiting severe abdominal cramps severe chill and fever (101 8 F) At 28 hours 105 cc of dark brownish red urine were obtained by catheter

Prominent oliguria azotemia hypochloremia and acidosis developed (table 8) The oliguria was severe for ten days (fig 3 Case 6) then a rise in urinary volume to 2950–3575 cc in 24 hours occurred on the thirteenth fourteenth and fifteenth days The urine specific gravity was low

During the first six days 2 500 cc of blood were given for the anemia and the hemoglobin concentration was elevated from 8 55 to 16 1 grams per cent The fluid intake during the first nine days approximated 6 750 cc The output of urine during this time was 635 cc Edema was not observed Sodium bicarbonate was given periodically (exact amount unknown)

Between the tenth and sixteenth days the fluid intake was less than the urinary output and naturally with the insensible loss the total output far exceeded the intake On the sixteenth day the urinary output dropped markedly and the patient had s vere convulsions Following intravenous fluid (dextrose in dis tilled water) and the increase and regulation of the dietary and oral water intake there was satisfactory recovery The patient was ambulatory on discharge from the hospital 21 days after the reaction

Comment The management in this case was essentially of three phase type but a complication occurred during the third phase as a result of insufficient water salt replacement

Early the anemia was corrected with 2 500 cc of blood During the renal in sufficiency phase there was no water salt overload On the contrary, less fluid was given for nine days than necessary to replenish the estimated insensible loss plus the scanty urinary output and the patient was actually slightly dehydrated during this time Nevertheless diuresis was prominent by the thirteenth day Failure to replace the water salt loss during the diuresis which was superimposed on an exist ing state of water deficit, apparently led to the decrease in urinary output and the convulsions The administration of fluids and a good dietary intake corrected these irregularities Recovery was satisfactory after these corrections

Case 7 This 48 year old white female entered the hospital for an operation of the left hip (arthoplasty) The pre operative hemoglobin concentration was 13 85 grams per cent Following operation the hemoglobin concentration dropped to 6 25 grams per cent During the five post operative days the patient was

TABLE 9—*Case 7*

Day	Blood		Plasma Prot	Hg B	Intake	Urine Vol	BP
	Urea N	Urea					
	mg /100 cc		*G/100 cc*	*G/100 cc*	*cc*	*cc*	*mHg*
1					4 200	60	
2					2 750	122	118/76
3	85	182	4 8	6 25	600	30	
4	100	214		7 2	3 000	75	
5					1 000	20	

given 2 500 cc of blood Later it was learned that the patient had received 2 or 3 transfusions seven years before each being followed by serious reactions

Following the first 500 cc of blood there was no overt reaction but the urinary output became scanty The second transfusion was followed by nausea fever and dark urine with much sediment Proteinuria was prominent

The oliguria persisted and the azotemia advanced (table 9) The patient expired 5 days postoperatively

Comment This case demonstrates the lethal effects of repeated incompatible transfusions Autopsy findings demonstrated the typical renal lesions of incompatible transfusion

Subsequently it was learned that the patient was D negative (Rh negative) and that D positive blood had been given At the time of these transfusions how ever Rh typing was not a routine procedure at this center but subsequently became so Without routine typing one can always expect an occasional case as this one

Case 8 A white female aged 42 years had a severe diffuse pneumonitis with a high remittent fever marked weakness and cyanosis The white blood cell count was 8 200 per cu mm with 74 per cent neutrophils The peripheral hemoglobin concentration was 14 45 grams per cent

One blood transfusion of 300 cc was given uneventfully Three days later a second blood transfusion was discontinued after 60 cc because of a severe reaction The following were noted sudden chest pain nausea vomiting cyanosis and a chill of 25 minutes duration Subsequently oliguria azotemia hypo-

chloremia acidosis and jaundice developed (table 10) The patient expired nine days later During this period the fluid intake far exceeded the urinary output

Comment This patient had an advanced pneumonitis of unknown etiology The incompatible transfusion added to complications which culminated in a fatal termination In view of present knowledge and the existence of pulmonary lesions the rather large fluid intake apparently had an added deleterious effect

TABLE 10 —*Case 8*

Day	Blood		CO_2 CP	Blood Cl	Hg B	Intake	Urine Vol	Icterus Index
	Urea N	Urea						
	mg /100 cc		*V %*	*mg /100 cc*	*G/100 cc*	*cc*	*cc*	
2	60	124	66			1 650	100	25
3	64 5	138	44			2 900	200	15
4	68	145	69	380	12 65	2 650	550	
5	82 5	[illegible]	70		13 25	3 700	150	

TABLE 11 —*Case 9*

Days	Blood		CO CP	Blood Cl	Serum Na	Serum K	Hg B
	Urea N	Urea					
	mg /100 cc		*V %*	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*
1–2	42	90	64	480	148	4 1	13 6
3–4	26	58	60	500	156	4 3	
5–6	30	66	70	520	152	4 8	11 85
7	28	60	64	480	140	5 1	

Intake	Urine Output	Total Urine Urea	Total Urine Cl
cc	*cc*	*Gm*	*Gm*
1200+	1800	5 6	4 5
3250	2300	2 3	2 3
3600	2300	7 5	1 2
1900	1600	12 8	1 6

Case 9 A white male aged 20 years of Type O cde/cde (Rh negative) received 100 cc of type A_1 D positive blood (probable type CDe/cDe) through an error The recipient experienced marked dyspnea a chill urticaria nausea vomiting and fever (temperature 100 8 F) Hemoglobinuria azotemia and a low urine specific gravity subsequently followed (table 11) Oliguria existed for two days following which the output increased to near 1000 cc for seven days At this time the patient was discharged Progress was satisfactory although recovery was not complete One month later he appeared well

During the renal insufficiency phase the intake was limited below the estimated insensible loss and urinary loss

Comment The antibody titer at the time of the transfusion in this case was known anti D agglutinating antibody 1 512 cryptagglutinoid type 1 2000 The incompatibility between the infused red cells and the patient s serum (antibodies) involved two antigens one of which was probably of homozygous type This

Comment The management in this case was essentially of three phase type but a complication occurred during the third phase as a result of insufficient water salt replacement

Early the anemia was corrected with 2 500 cc of blood During the renal insufficiency phase there was no water-salt overload On the contrary less fluid was given for nine days than necessary to replenish the estimated insensible loss plus the scanty urinary output and the patient was actually slightly dehydrated during this time Nevertheless diuresis was prominent by the thirteenth day Failure to replace the water salt loss during the diuresis which was superimposed on an existing state of water deficit apparently led to the decrease in urinary output and the convulsions The administration of fluids and a good dietary intake corrected these irregularities Recovery was satisfactory after these corrections

Case 7 This 48 year old white female entered the hospital for an operation of the left hip (arthoplasty) The pre operative hemoglobin concentration was 13 85 grams per cent Following operation the hemoglobin concentration dropped to 6 25 grams per cent During the five post operative days the patient was

TABLE 9 --*Case 7*

Day	Blood		Plasma Prot	Hg B	Intake	Urine Vol	BP
	Urea N	Urea					
	mg /100 cc		G/100 cc	G/100	cc	cc	mHg
1					4 200	60	
2					2 750	122	118/76
3	85	182	4 8	6 25	600	30	
4	100	214		7 2	3 000	75	
5					1 000	20	

given 2 500 cc of blood Later it was learned that the patient had received 2 or 3 transfusions seven years before each being followed by serious reactions

Following the first 500 cc of blood there was no overt reaction but the urinary output became scanty The second transfusion was followed by nausea fever and dark urine with much sediment Proteinuria was prominent

The oliguria persisted and the azotemia advanced (table 9) The patient expired 5 days postoperatively

Comment This case demonstrates the lethal effects of repeated incompatible transfusions Autopsy findings demonstrated the typical renal lesions of incompatible transfusion

Subsequently it was learned that the patient was D negative (Rh negative) and that D positive blood had been given At the time of these transfusions however Rh typing was not a routine procedure at this center but subsequently became so Without routine typing one can always expect an occasional case as this one

Case 8 A white female aged 42 years had a severe diffuse pneumonitis with a high remittent fever marked weakness and cyanosis The white blood cell count was 8 200 per cu mm with 74 per cent neutrophils The peripheral hemoglobin concentration was 14 45 grams per cent

One blood transfusion of 300 cc was given uneventfully Three days later a second blood transfusion was discontinued after 60 cc because of a severe reaction The following were noted sudden chest pain nausea vomiting cyanosis and a chill of 25 minutes duration Subsequently oliguria azotemia hypo-

The patient was found to be D negative and showed antibodies in his serum There was no renal insufficiency and there were no sequelae

Case 1 A 28 year old white female was given a transfusion following an operation She had delivered an infant five months before The patient was of type A_1 D negative and received 400 cc of A_1 D positive blood There was a severe chill and the temperature rose to 104 2 F within three hours No renal insufficiency developed and there were no apparent sequelae

Case 18 The patient was of Type B and received 100 cc of type A blood A chill and fever resulted but there were no other abnormalities noted No renal insufficiency developed in the subsequent four days An operation within a week was followed by fatal pulmonary embolism The patient lived for two days with this complication and during this period renal insufficiency was prominent

Comment The hemolytic reaction did not yield any discernible renal damage It is doubtful if the subsequent renal insufficiency, which followed a prolonged period of hypotension was directly related to the transfusion reaction

RENAL INSUFFICIENCY DUE TO OTHER CAUSES

Acute renal damage with temporary renal insufficiency may occur from various causes Such cases may resemble closely those of incompatible blood transfusions and they may be similar to those of the transfusion groups Since a similar type of management seems applicable 10 illustrative cases are presented in support of the discussion on the transfusion group

A *Prolonged Hypotension* Four cases of prolonged hypotension followed by renal insufficiency are included

Case 1 A white female age 32 years entered the hospital for delivery of a full term infant When dilatation was complete a saddle block anesthesia was given The BP which was 118/70 promptly dropped to 80/50 mm Hg The pressure ranged between 70/40 and 92/50 until delivery of a stillborn infant At this time it became lowered to 60/40 and then to imperceptible levels

The uterus failed to contract profuse bleeding continued and the BP remained imperceptible for one hour when a hysterectomy was performed

For an additional 50 hours the BP fluctuated between 50/38 and 80/50 During this period the patient received 4 500 cc of blood and 1 500 cc of dextrose solution intravenously The post shock period began at this time when the BP became 120/80 mm Hg

Table 12 contains data on this case Prominent azotemia acidosis hypochloremia hyponatremia and oliguria developed The BP became slightly elevated

During the first eight days the patient received about 28 840 cc of fluids while at the same time the urinary output totaled 5 495 cc Of the intake 10 200 cc consisted of 5 per cent dextrose solution by vein and approximately 11 390 cc was water by mouth Marked restlessness and irregular muscular jerks and twitching developed within five days On the sixth day the patient was irrational groaned frequently and was comatose There were generalized edema profuse sweating salivation and large watery stools The clinical picture was that of water intoxication

One of us first saw this case on the seventh day At this time there was a low serum sodium (118 mEq/L) and a high serum potassium (10 5 mEq/L) concentration Lowering of the hyperpotassemia became paramount Accordingly 20 grams of sodium bicarbonate were given by stomach tube The Serum K concentration dropped away from danger levels and the Serum Na concentration was elevated

During the ensuing 72 hours (seventh eighth, and ninth days) the fluid intake was limited to negligible quantities The patient was given 130 cc of 5 per cent NaCl by vein as two doses and an additional 6 grams of sodium bicarbonate (and calcium gluconate) Convulsions occurred for almost a 24 hour period These ceased after one dose of magnesium sulfate (4 cc 25 per cent by muscle) The patient remained in deep coma until she expired on the fourteenth day following the shock

Oliguria remained for eight days following which there was a daily increase in urinary output By

multiple antigen antibody relationship might well have accounted for the violent nature of the hemolysis (Hill Haberman Jones concept) and thereby possibly in fluencing the degree of renal damage Since the recipient was an Rh antibody donor anemia or other complications were not present at the time of the transfusion

Case 10 A white male aged 46 years had a bleeding peptic ulcer He had received a transfusion six teen years before Prior to admission to this hospital 3 transfusions had been given The third transfusion was followed by a severe reaction with a chill hemoglobinuria and oliguria for four days

It was determined that the patient was D negative (Rh negative) Following transfusions of D nega tive blood the patient was discharged improved Renal damage apparently was mild and recovery was rapid and satisfactory

Case 11 This 64 year old white male suffered from a debilitating febril- dis as subs qu.ntl found to be mycosis (autopsy) The patient was of typ. O D positive Through an error he was given blood of Type A_1 D positive Following 100 cc a severe reaction developed which was characterized by nausea vomiting chill and fever (temperature 98 6 to 102 6 F) Th- first urine sp cim n was dark red A urine sp cimen 23 hours later was dark brown in color

Within one and one half hours after the reaction the patient was given 500 cc of compatible blood and 500 cc of on sixth molar sodium lactate solution No renal insufficiency developed in this case and there were no apparent sequelae from the reaction

Comment All seemingly severe incompatible (hemolytic) blood transfusion re actions are not followed by renal insufficiency as this case attests The anemia was only moderate and no hypotension ensued One wonders if these factors and the immediate transfusion were concerned in absence of renal insufficiency

Case 12 A white male aged 71 years had a diagnosis of hypogranulocytosis and bone marrow de pression anemia (sulfanamide etiology) A successful transfusion of 500 cc was given Two days later a second apparently compatible transfusion was attempted After 50 cc of blood were given there devel oped a severe chill dyspnea cyanosis and the temperature reached 105 F The patient became jaundiced during the next hours

No hemoglobinuria was detected There was no renal insufficiency and no sequelae were noticed

The next 4 cases have been previously reported in a discussion on Rh iso-immuni zation [36] They occurred prior to the establishment of the routine Rh typing of all bloods in this center Only brief statements concerning each case are given

Case 13 A white male age 38 years had received three transfusions for anemia Subsequently the first transfusion in this hospital was uneventful The second one was followed by a severe chill and fever It was l-arned that the latter involved D positive (Rh positive) blood while the patient was D negative There were no sequelae

Case 14 A white male had a transfusion elsewhere prior to admission A severe reaction occurred after 250 cc of blood were given here The patient was D negative the infused blood was found to be D posi tive Antibodies developed in the patient s serum There were no sequelae

Case 15 A white female age 41 years received 10 transfusions in this hospital The first transfusion was uncomplicated the second one was followed by a chill after 400 cc had been given The third trans fusion was discontinued after 150 cc because of a severe reaction The chill lasted 40 minutes

The patient was found to be D negative (Rh negative) and developed antibodies in her serum There were no renal sequelae

Case 16 A white male age 37 years had a colectomy in 1943 for polyposis One transfusion had been given in 1942 A transfusion prior to the present admission was followed by a chill fever and headache Two other transfusions on the present admission were associated with reactions The third transfusion was succeeded by a chill fever hemoglobinuria and jaundice the following day

Severe oliguria existed for five days then mild oliguria was present for three more days Azotemia and hypochloremia accompanied the oliguria (table 13) During this period there was a tendency toward alkalosis which became true alkalosis by the eighth day All factors concerned with the alkalosis were not fully understood even after complete recovery The onset of alkalosis was associated with a temporary recession of the urine volume The urine volume fluctuated between oliguria and normal value during the alkalotic period Copious diuresis occurred on the twenty third day the CO_2 combining power of plasma being normal from this time on

During the first eight days the fluid intake far exceeded the urinary output The estimated insensible loss accounted for a portion of the extra intake The patient became irrational restless talkative displayed irregular muscular movements and subsequently was comatose The irrational loud talk continued until the fifteenth day at which time she became more rational At this time the blood urea concentration

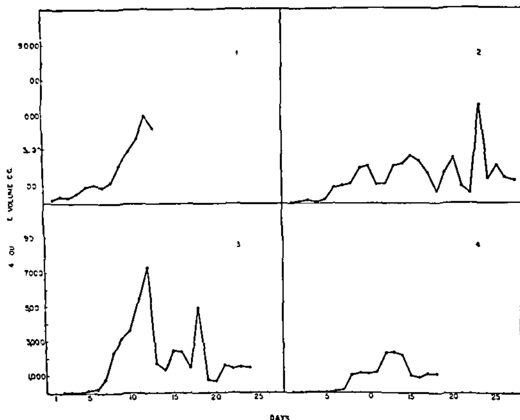


FIG 4 THE OLIGURIA DIURESIS CURVES OF THE FOUR CASES IN THE PROLONGED HYPOTENSION GROUP

decreased to near normal the blood chloride and serum sodium concentration became normal but the alkalosis appeared and the urine volume receded once more

During the first sixteen days 60 to 75 per cent of the fluid intake was by vein and 11 000 cc contained isotonic sodium salt (70 per cent NaCl) Between the nineteenth and twenty fourth day (alkalosis period) the patient was given 7 000 cc of 0 82 per cent NH_4Cl solution and 480 cc of 25 per cent human albumin solution by vein Copious diuresis occurred at this time following which there was recovery Between the seventeenth and twenty sixth days the urinary output plus the estimated insensible loss far exceeded the fluid intake

There were 3 incidents of recurring oliguria between the tenth and twenty second days (fig 4 Case 2) Alkalosis existed on each occasion During the second incident fluid accumulated in the pleural space requiring thoracentesis In the third incident there was atelectasis requiring bronchial aspiration At this time the BP was temporarily lowered (85/55) and the patient had a convulsion Muscular twitching

the twelfth day there was copious diuresis Substantial quantities of urea were cleared from the body at this time but the blood urea concentration remained elevated The urine specific gravity varied between 1 007 and 1 012 throughout the course The salts lost in the urine were replenished by vein

The CO_2 combining power of plasma the blood chloride and serum sodium concentrations were elevated to normal range during the course A low potassium concentration was being corrected by KCl by stomach tube The generalized edema disappeared

The autopsy findings demonstrated diffuse cerebral and cerebellum damage with myriads of punctate hemorrhages Microscopically chromatolysis karyolysis necrosis and neuronophagia were noticed The distal renal tubules were everywhere lined by flattened epithelium (regeneration)

TABLE 12 —*Case 1*

Day	Blood		CO_2 CP	Blood Cl	Serum Na	Serum K	Hg B
	Urea N	Urea					
	mg /100 cc		1 %	mg /100 cc	mEq/L	mEq/L	G/100 cc
2	68	146	50	480			10 1
4	111	236		400			13 4
6	103	221		420			
8	140	299	31	440	118	10 5	15 8
9	136	291	40	410	125	6 1	
11	165	355	42	470	137	5 0	
12	170	370	49	500	137	4 1	9 15
13	150	330	54	520	153	2 3	
14	220	470	46	520			13 4

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl	Total Urine N	Total Urine K	BP
	c		Gm	Gm	Gm	Gm	mHg
1– 2	10 320	545					
3– 4	6 4-0	815					140/60
5– 6	8 300	2 025					154/100
7– 8	3 800	2 110					
9	50	2 200	12 9	4 2			
10–11	3 160	6 -00	53 6	21 9			
12	5 500	5 000	46 4	23 4	9 7	3 3	
13	5 100	4 300	38 4	15 5			

Comment The case was one of acute renal insufficiency following hypotension The patient was overhydrated and developed water intoxication A hyperpotassemia was quickly corrected

The oliguria-diuresis curve (fig 4 Case 1) in this case was quite similar to that of the incompatible transfusion cases It is considered that the renal phase in this case was recovering satisfactorily at the time of death The diffuse brain damage was overwhelming The appearance of the kidneys showing prominent tubular regeneration was in keeping with the clinical progress of renal function

Case 2 A white female aged 45 years was in a car accident 30 minutes prior to admission She sustained a crushed chest head injury and apparently multiple internal injuries On arrival the BP was imperceptible The dextrose solution and 500 cc of blood elevated the pressure to 80/52 within 4 5 hours By 9 hours the BP was 1-0/80 pulse rate 120

Severe oliguria existed for five days then mild oliguria was present for three more days Azotemia and hypochloremia accompanied the oliguria (table 13) During this period there was a tendency toward alkalosis which became true alkalosis by the eighth day All factors concerned with the alkalosis were not fully understood even after complete recovery The onset of alkalosis was associated with a temporary recession of the urine volume The urine volume fluctuated between oliguria and normal value during the alkalotic period Copious diuresis occurred on the twenty third day the CO_2 combining power of plasma being normal from this time on

During the first eight days the fluid intake far exceeded the urinary output The estimated insensible loss accounted for a portion of the extra intake The patient became irrational restless talkative displayed irregular muscular movements and subsequently was comatose The irrational loud talk continued until the fifteenth day at which time she became more rational At this time the blood urea concentration

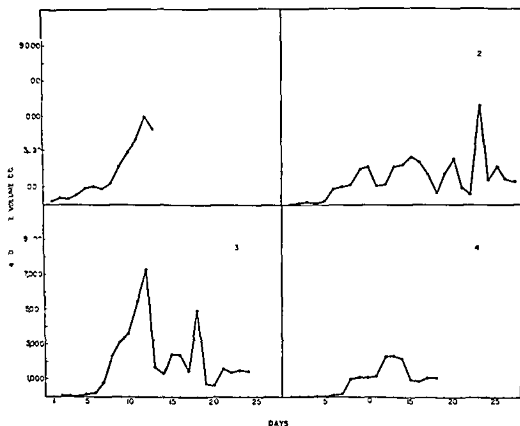


FIG 4 THE OLIGURIA DIURESIS CURVES OF THE FOUR CASES IN THE PROLONGED HYPOTENSION GROUP

decreased to near normal the blood chloride and serum sodium concentration became normal but the alkalosis appeared and the urine volume receded once more

During the first sixteen days 60 to 75 per cent of the fluid intake was by vein and 11 000 cc contained isotonic sodium salt (70 per cent NaCl) Between the nineteenth and twenty fourth day (alkalosis period) the patient was given 7 000 cc of 0 82 per cent NH_4Cl solution and 480 cc of 25 per cent human albumin solution by vein Copious diuresis occurred at this time following which there was recovery Between the seventeenth and twenty sixth days the urinary output plus the estimated insensible loss far exceeded the fluid intake

There were 3 incidents of recurring oliguria between the tenth and twenty second days (fig 4 Case 2) Alkalosis existed on each occasion During the second incident fluid accumulated in the pleural space requiring thoracentesis In the third incident there was atelectasis requiring bronchial aspiration At this time the BP was temporarily lowered (85/55) and the patient had a convulsion Muscular twitching

periodic cyanosis and mental cloudiness continued until the twenty fourth day (diuresis) After this time recovery occurred The urine specific gravity was low (1 005–1 012)

Comment This was a highly complicated case The renal insufficiency phase was progressing in the usual manner until the tenth day The development of alkalosis seemed to alter the progress Alkalosis[37] has been considered to cause renal damage and insufficiency Finally a pulmonary complication with hypotension interfered with the urinary output Recovery occurred after a late copious diuresis (twenty third day)

TABLE 13 —*Case 2*

Day	Blood		CO CP	Blood Cl	Serum Na	Plasma Prot	Hg B
	Urea N	Urea					
	mg /100 cc		[illegible]	*mg /100 cc*	*mEq/L*	*G/100 cc*	*G/100 cc*
2	29	61	54	480		5 8	
4	59	127	53	430		7 6	11 8
8	83	178	83	390		5 4	15
12	71	153	84	500		5 8	12 35
16	24	52	90	500	141	5 1	13 25
18	26	56	91	480	161	5 1	
20	43	92	100	470	145 6	5 3	
22	26	54	80	500	158	5 4	12 35
26	19	42	72			5 6	11 75

Days	Intake	Urine Vol
		cc
1– 2	7 800	290
3– 4	2 990	350
5– 8	14 380	5 220
9–12	9 470	7 060
13–16	15 295	8 610
17–18	5 600	3 440
19–20	4 150	2 945
21–22	4 940	6 725
23–26	11 630	5 830

There was an excessive fluid and salt intake during the first sixteen days This water salt overload appeared to be an additional complication in the case

It is of interest that repeated doses of aminophyllin and salyrgan in early phases did not appear to alter the urinary output

Case 3 A 57 year old white male complained of severe epigastric pain and pain in both arms of 20 hours duration He had had severe hypertension for seven years The BP on admission was 200/170 mm Hg Coronary occlusion and cholecystitis with cholelithiasis were suspected The latter proved correct during the first day

On the first day the patient had a chill the temper ture was elevated to 104 F and later he became jaundiced During the second day the temperature was subnormal (97 F) and the BP was lowered to 120/90 for several hours There was coffee ground vomitus and subsequent tarry stools The patient developed oliguria severe azotemia acidosis hypochloremia and hyponatremia (table 14)

For seven days there was extreme oliguria (fig 4 case 3) During this time the fluid intake far exceeded the urinary output and 53 per cent of the intake consisted of intravenous dilute plasma (salt containing) The patient became irrational moaned and groaned and talked loudly These manifestations disappeared with the diuresis

After the seventh day the urinary output increased each day The peak of the copious diuresis occurred on the twelfth day There was a concomitant recession of the azotemia and mental confusion From this time on the fluid intake matched the urinary output plus the estimated insensible water loss The patient recovered from the acute renal insufficiency The urine specific gravity was depressed throughout and after recovery (1 004–1 010)

TABLE 14 —*Case 3*

Day	Blood		CO_2 CP	Blood Cl	Serum Na	Hg B
	Urea N	Urea				
	mg /100 cc		*V %*	*mg /100 cc*	*mEq/L*	*G/100 cc*
2						
4–6	130	290	38	460		
6	170	380	36			
7	187	398			122	8 55
11	140	300	79			
14	80	175	72			
16	60	125	63			
20	40	86				

Days	Intake	Urine Vol	Proth	Icterus Index
	cc	*cc*	*%*	
1– 2	1 500	0		71
3– 5	4 675	150	33	62 5
6	3 100	200	70	30
7	4 100	800	100	27
8–11	15 150	9 180		
12–14	12 450	10 400		
15–16	7 950	4 900		13
17–20	6 250	8 900		

Comment The renal insufficiency in this case was related to the relative hypotension and perhaps jaundice The course of the renal phase (oliguria and recovery) was quite similar to that of cases with incompatible transfusion

During the first week there was a moderate water salt overload with mental symptoms This did not appear to delay appreciably the usual appearance of the diuresis Aminophyllin did not seem to accelerate the renal output

Case 4 A white female aged 23 years had pyloric obstruction with nausea and vomiting for two months She was primarily a nutritional problem

For one week the stomach was drained by suction and the fluid obtained was matched by parenteral intake Then for 60 hours the patient was on a high amigen and dextrose intake by vein Five liters of amigen had been given then after 100 cc of another batch the patient developed a slight chill cyanosis rapid respiration and the pulse and blood pressure became imperceptible The superficial veins were distinct the skin was warm (103 F) and the patient had an involuntary stool Next there were generalized erythema nausea and vomiting and the patient coughed blood For 12 hours the blood pressure was low usually imperceptible to 80/40 and for an additional seven days it fluctuated between 80/50 and 96/80

There was anuria for 33 hours (table 15) During this period the patient received by vein 450 cc of concentrated plasma (4 X Normal) 3000 cc of 5 per cent dextrose and 500 cc of blood During the following 70 hours 4 000 cc of blood and 2 000 cc of dextrose solution were given by vein The hemoglobin concentration of peripheral blood was elevated from 10 8 to 18 65 grams per cent Fluid accumulation in the right pleural space required periodic thoracentesis After the last transfusion there was respiratory difficulty restlessness cyanosis distended neck veins pulmonary rales lowered BP and weak pulse Improvement occurred after 1400 cc of blood were withdrawn

The urinary output increased after the seventh day (fig 4 Case 4) and the azotemia abated The urine specific gravity range was 1 006–1 013 On the ninth day a jejunostomy was performed and the patient was fed by this means Twenty eight days after the original hypotension a gastro-jejunostomy was performed because of fibrous pyloric obstruction

TABLE 15 —*Case 4*

Day	Blood		CO_2 CP	Blood Cl	Serum Na	Serum K	Plasma Prot	Hg B
	U ea N	Urea						
	mg /100 cc		V %	*mg /100 cc*	*mEq/L*	*mEq/L*	*G/100 cc*	*G/100 cc*
3	65	140	53	550	135	6 1		10 8
5	91	195		520	141	5 8		15 8
7	107	229	50		146	4 9	5 8	18 65
12	46	99			146	5 1		12 2
15	21	46						
21	32	67						

Days	Intake	Urine Vol	Tot l Uri e Urea
	cc	*cc*	*Gm*
1– 3	6 150	100	
4– 5	2 560	1 150	
6– 7	1 600	2 150	
8–12	2 500	8 525	
13–15	6 000	3 000	
22		2 480	61

Comment Renal insufficiency followed a period of hypotension The hypotension was related to amigen administration and an acute pulmonary complication (infarct?) Subsequently there was vascular overload by blood transfusion which was relieved by blood withdrawal

The urinary output began to increase on the seventh day The azotemia was prominently lowered by the sixteenth day The recovery period and other features resembled the other cases

B *Renal Insufficiency Following Transurethral Prostatic Resection* It has been reported[38, 39] that transurethral prostatic resection using distilled water as the irrigating fluid may be followed by severe hemolysis jaundice and renal insufficiency Presumably this is a distilled water type of hemolysis The course of these cases is not unlike that of the incompatible transfusion cases Four of these cases are briefly presented

Case 1 This case is being reported and discussed by Baird and Spence [40] We are indebted to these workers for the herein mentioned pertinent information

Renal insufficiency became evident soon after a transurethral prostatic resection (age 72) During the first eight days there was prominent oliguria The intravenous fluid intake during this period was 25 000 (dextrose solution mainly) while the corresponding urinary output was 1100 cc The blood urea was elevated to 199 mg per 100 cc The patient became edematous irrational groaned and moaned and displayed muscular twitching

The urinary output began increasing on the ninth day and copious diuresis appeared on the following day (fig 5 Case 1) The 24 hour urine output was 10 650 cc on the eleventh day and 10 800 cc on the twelfth day At the same time the fluid intake on the eleventh day was 1600 cc and the twelfth day 3 830

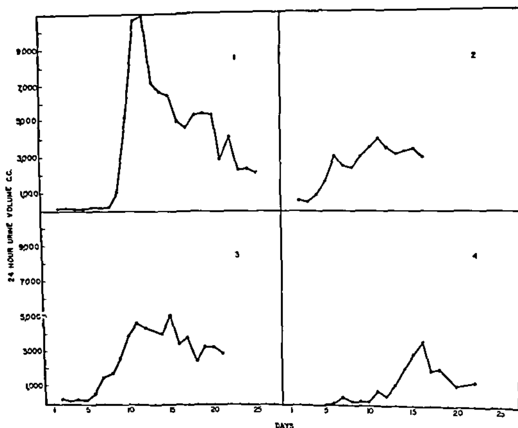


FIG 5 THE OLIGURIA DIURESIS CURVES OF THE FIRST THREE CASES OF RENAL INSUFFICIENCY FOLLOWING TRANSURETHRAL PROSTATECTOMY (1 2 3)
Case 4 is the case of CCl_4 poisoning with renal insufficiency

cc On the twelfth day there were two grand mal convulsions The patient improved promptly as soon as the fluid intake was increased During the following seven days the daily intake amounted to 6 000 to 8 000 cc Improvement was rapid and by the twenty first day the blood urea concentration was o mg per cent

Comment The oliguria-diuresis curve o the case mimics closely that of the transfusion cases Apparently some fluid overload occurred during the first week and a water salt deficit during the extremely copious diuresis The convulsions apparently were due to dehydration during the third phase Correction of this deficit was followed by prompt recovery

Ca Thi ase is likewise being reported in detail by Baird and Spence [40]

F llov ing a transurethral prostatic resection oliguria and azotemia developed (Blood urea 190 mg

per 100 cc) The oliguria lasted four days and during this period there was a guarded fluid intake By the ninth day there was prominent diuresis At this time the fluid intake was accelerated to cope with the output Recovery was satisfactory (fig 5 Case 2)

Comment In this case the difficulties of Case 1 were averted by limiting the fluid intake until the onset of the diuresis In essence this was a three phase manage ment

Case 3 With this case we had the opportunity to initiate the three phase management at the outset of the renal insufficiency

A 67 year old white male had a transurethral prostatic resection during which 17 grams of tissue were removed During the operation the blood pressure remained normal (140/70 mm Hg) and the patient

TABLE 16 —*Case 3*

Day	Blood		CO_2 CP	Blood Cl	Serum Na	Serum K	Hg B
	Urea N	Urea					
	mg /100 cc		[illegible]	mg /100 cc	mEq/L	mEq/L	G/100 cc
2	72	154					11 15
5	120	250	56	450	122	4 7	
8	125	265	55	480	130	4 1	15 0
10	150	320	50	470	138	4 3	
13	130	270	55	480	148	5 9	
15	60	130	58	490	136	3 23	12 2
17	33	71	64	500	140	5 7	
20	15	32					

Days	Intake	Urine Vol	Urine Urea Conc	Urine Cl Conc	BP
	cc	cc	mg /100 cc	mg /100 cc	mHg
1– 2	5 200	300			60/30
3– 5	2 400	620	500	363	134/80
6– 8	5 000	4 025	517	222	132/80
9–10	6 700	6 700	1 062	250	160/80
11–13	14 580	13 400	708	260	170/78
14–15	11 350	9 300	267	220	
16–17	4 850	7 400	178	160	
18–20	9 550	9 150	222		

received no blood transfusion On returning from the operating room the blood pressure was 132/84 pulse rate 96 The usual continuous irrigation of the bladder was in progress using the Foley catheter Within 3 5 hours the blood pressure became 90/60 then 70/50 and the pulse rate was elevated to 140 The patient became cyanotic The respirations were labored and coma developed A cerebral accident was suspected but no localizing neurological signs could be elicited The blood pressure remained depressed (60/30–80/40) for 17 hours Then it rose to 90/60 and 100/70 for 48 hours After this period the pressure was elevated to 160/80 Slight jaundice appeared within 24 hours

Renal insufficiency with oliguria azotemia and hyponatremia developed (table 16) The oliguria was *severe for six days Then there was a gradual elevation of the urinary output until a copious diuresis* occurred on the eleventh day (fig 5 Case 3) The urine specific gravity remained low Recovery was quite rapid from this time on

Management during the renal insufficiency phase consisted of four steps (1) nearly complete replace ment of the estimated insensible water loss and urinary output (2) the administration of small doses of

sodium bicarbonate to cope with hyponatremia and to prevent an excessive drop in the blood pH (3) A fluid intake mainly of a high caloric low salt formula by mouth (4) Water soluble vitamins by intra venous route daily No generalized edema or mental cloudiness developed The patient read the daily papers conversed freely with all visitors and was in good spirits at all times (Blood urea 320 mg per cent)

The 5 200 cc of fluids given during the first and second days consisted of blood and plasma intra venously for combating the hypotension During the first ten days the total fluid intake fell somewhat below the output (estimated insensible loss plus urinary output) The slight water deficit was main tained purposely since the patient developed scattered basal rales It is extremely interesting that despite this dehydrated state diuresis nevertheless occurred at the expected time (eleventh day)

The urine urea and chloride concentrations were not determined each day but sufficiently close deter minations were performed to give a good estimate of the urinary output of those two ingredients (table 16) With the onset of the diuresis and the first phases of recovery the urine urea concentration was prominently elevated Substantial quantities of urea were cleared from the body thus accounting for the recession of the azotemia At the time the urine chloride concentration (as NaCl) remained essentially unaltered but due to the mounting urine volume greater and greater amounts of salt were discarded from the body As in the incompatible transfusion cases salt replacement was instituted at this time

The two sodium-containing salts given by mouth were sodium bicarbonate and sodium chloride Table 17 contains the amounts given

During the first seven days $NaHCO_3$ was given to combat the hyponatremia and to bolster the down ward trend of the alkali reserve On the eighth day at the onset of the diuresis NaCl was added to the

TABLE 17

	Day										Total
	4	5	6	7	8	9	10	11	12	13	
$NaHCO_3$ Gm	2	4	2	4	9	8	10	15	7 5	5	66 5
NaCl Gm					4	4	10	15			33 0

intake The total replacement of sodium approximated a gram per gram basis Thus up to the thirteenth day the total urinary sodium output was estimated to be 25 grams and the oral intake amounted to 30 9 grams In actual practice this replacement was conducted on a 12 hour basis Water and salt were given according to the urinary volume salt content and the level of the plasma CO combining power serum sodium and chloride concentrations

The results with the above management were most encouraging On the fifteenth day the patient was placed on a general diet and was allowed to adjust his own intake and output Progress continued satis actorily and on the twentieth day the blood urea concentration was 32 mg per 100 cc

Comment This case was one of severe renal insufficiency following a transurethral prostatic resection the usual continuous bladder irrigation and a prolonged period of hypotension The patient had signs of generalized senile arteriosclerosis

The three phase management was associated with a remarkable recovery com parable to that of incompatible transfusion cases

Case 4 A 73 year old white male developed a chill and shocklike picture (hypotension tachycardia sweating mental depression) soon after a transurethral prostatic resection where water was used as irrigating fluid A 500 cc blood transfusion was given during the resection Jaundice became definite during the following day A carefully collected sample of plasma shortly after the resection revealed a prominent hemoglobinemia (826 mg of free hemoglobin per 100 cc of plasma) During this first phase 1500 cc of blood and 60 cc of salt poor concentrated albumin (25 per cent) were given intravenously and the BP reached 90 mm Hg

Renal insufficiency developed as evidenced by azotemia Hypochloremia and hyponatremia also appeared (table 18)

The three phase management was instituted recovery was satisfactory and the patient was discharged on the twenty sixth day During the renal insufficiency phase the total urinary volume plus the estimated insensible loss far exceeded the measured intake Yet diuresis and recovery were quite satisfactory

C *Carbon Tetrachloride Poisoning*

A 31 year old white female was in the habit of consuming large quantities of alcohol Six days prior to admission she was exposed to CCl_4 fumes from a broken fire extinguisher Nausea vomiting epigastric pain jaundice and renal insufficiency de eloped

TABLE 18 —*Case 4*

Day	Blood		*CO CP*	*Blood Cl*	*Serum Na*	*Serum K*	Icterus Index	*Hg B*
	Urea N	Urea						
	mg /100 cc		*V %*	*mg /100 cc*	*mEq/L*	*mEq/L*		*G/100 cc*
2	60	128	52	320	136	6 3	133	9 7
4	89	190	52	580				
6	77	165	66	520	132	6 1		10 4
8	58	125	58	500				
12	51	110	70	480	148			
15	37	80						

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl
	cc	*cc*	*Gm*	*Gm*
1– 2	4 760	2 150		
3– 4	6 000	4 875	49 6	6 9
5– 6	8 300	7 900	57 6	9 5
7– 8	7 000	8 000	29	18 4
9–12	13 000	14 800	73 2	34
13–15				

Oliguria was severe for twelve days during which time there was a mounting azotemia (table 19) During the period the fluid intake far exceeded the urinary output (80 per cent of intake consisted of 5 per cent dextrose solution by vein) The patient developed generalized edema and had persistent nausea and vomiting The urinary output began mounting on the thirteenth day and reached its peak on the sixteenth day (fig 5 Case 4) A low specific gravity was noted throughout (1 004–1 012) The urinary output then far exceeded the intake for a while Recovery followed the diuresis

Comment There was water overload during the first ten days The peak of the diuresis occurred later than in the preceding cases without this complication

D *Extensive Burns*

A white male aged 43 years sustained burns involving an estimated 85 per cent of the body surface The patient was in se ere shock for over six hours He received 1500 cc of blood 750 cc of concentrated plasma (3 X Conc) 720 cc of concentrated albumin solution (25 per cent) and 6750 cc of dextrose in distilled water during the first 48 hours Hemoconcentration persisted for 30 hours (*Hgb 19–21 grams per cent*) and then abated Oliguria and renal insufficiency were evident by the second day The mental

status began deteriorating on the third day delirium appeared and the patient died on the ninth day after two bouts of pulmonary embolism

The renal insufficiency aspect of this case was treated by the three phase type of management (table 20) Renal recovery was well advanced at the time the patient died as demonstrated by daily urine volume of 3000 cc with 30 grams of urea Despite an adequate fluid intake the patient continued to exhibit signs of extracellular dehydration and a mounting blood urea concentration

Autopsy findings revealed evidence of severe brain damage The distal renal segments were lined mainly by flattened epithelium Focal inflammatory foci were seen in the kidney sections Generalized edema was not observed

TABLE 19

Day	Blood		Hg B	Days	Intake	Urine Vol	Icterus Index
	Urea N	Urea					
	mg /100 cc		G/100 cc		cc	cc	
6	79	169	10 8	6	2 000	70	22
10	90	200		7–10	8 200	1 255	24
13	90	200		11–13	8 500	2 540	
17	75	155		14–17	2 450	10 655	
25	30	62					

TABLE 20

Day	Blood		CO_2 CP	Blood Cl	Serum Na	Serum K	Hg B
	Urea N	Urea					
	mg /100 cc		V %	mg /100 cc	mEq/L	mEq/L	G/100 cc
2					167	6 3	19
4							
6	70	150	46	300	148	7 3	14 6
7	140	300		420			15
9	163	350	45	400	145	5 6	10 1

Days	Intake	Urine Vol	Total Urine Urea	Total Urine Cl
	c	cc	Gm	Gm
1–2	9 885	575		
3–4	5 825	985		
5–6	6 100	2 100	19 6	1 8
7	2 590	1 500	10 7	1 2
8–9	11 875	6 000	60	3 6

Comment This case resembles closely Case 1 of the Prolonged Hypotension group Expiration, apparently resulting from central damage, occurred despite a recovering renal function Again a mounting blood urea concentration associated with an adequate fluid intake and urinary output appeared as a bad prognostic sign

DISCUSSION

1 *The Oliguria Diuresis Curve*

In Figures 3, 4 and 5 the urine volume in cc is plotted against the time in days It will be noted that the general configuration of these curves is the same in all

Renal insufficiency developed as evidenced by azotemia Hypochloremia and hyponatremia also appeared (table 18)

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4	89	190	52	580				
6	77	165	66	520	132	6 1		10 4
8	58	125	58	500				
12	51	110	70	480	148			
15	37	80						

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13–15				

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2 *Urine Urea vs Chloride Concentration*

Another interesting finding is depicted in Fig 6 namely the relation of the urine urea and chloride concentration (as NaCl) as cases progressed Cases 1 2 and 3 recovered successfully Case 4 expired during renal insufficiency It will be noted that during the renal insufficiency phase the concentration of these two substances is similar The urea concentration was frequently 5–20 per cent of normal With the onset of diuresis and recovery the urine urea concentration usually increased appreciably over the chloride concentration (Cases 1 2 and 3) In Case 4 renal insufficiency persisted and the two concentrations remained near each other

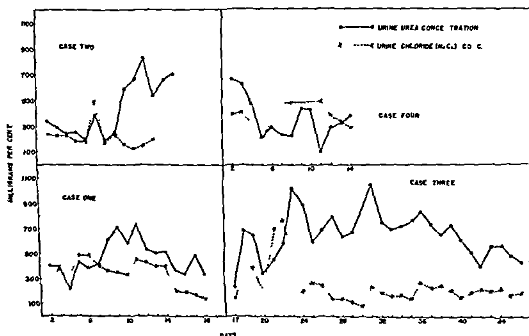


Fig 6 The Relationship of Urine Urea and Chloride (as NaCl) Concentration in the Urine during the Course of Four Cases of Renal Insufficiency following Hemolytic Transfusion Reaction

This observation has been made on 10 cases The data suggest that with recovery the urine urea concentration is elevated (40–75 per cent of normal) and that the chloride concentration remains less disturbed The relationship of urine urea and chloride appears to be of prognostic value

3 *General Comment*

Death following incompatible transfusions most commonly results from renal insufficiency and associated complications The present study indicates that the frequency of renal insufficiency may not necessarily be high Thus of 18 cases having a prompt hemolytic reaction only 55 5 per cent developed demonstrable renal insufficiency This observation is in keeping with the deduction that factors other than hemolysis per se are necessary for the inducement of evident renal damage The presence of certain complications in 9 of the 10 cases developing renal insufficiency supports this contention In 5 cases there had been prominent hemor-

cases For a while the urine output is low (renal insufficiency phase) then there is a gradual increment leading to a copious output (diuresis phase) These characteristic curves may be termed the oliguria diuresis curves

In Fig 3 are represented cases of incompatible (hemolytic) transfusion reactions in Fig 4 cases of prolonged hypotension and in Fig 5 3 cases following transurethral prostatectomy (1, 2 3) and 1 case of CCl_4 poisoning (Case 4)

A study of these curves yields significant information At once the similarity between the various cases is evident The period of renal insufficiency (oliguria) began to abate between the fifth and tenth day (average of 7 2 days) A gradual increment in urine volume followed until a peak of diuresis was reached The peak was reached between the tenth and eighteenth day (average 12 4 days) These were constant findings which seemed to indicate the time necessary for recovery of damaged renal elements The constancy of this curve has been found to be of much help in evaluating the progress and the prognosis of a case

A study of the oliguria diuresis curve from various types of cases has demonstrated an inability of various therapeutic procedures to alter its characteristic appearance Thus forcing fluids forcing salts giving large volume of an alkalinizing substance such as sodium lactate the use of various diuretics (as salyrgan) intravenous aminophyllin and sodium sulfate solutions did not encourage early diuresis Cases have been reported in which a therapeutic procedure was instituted between the eighth and twelfth day and because diuresis commenced at this time, the procedure was given credit for initiating it In evaluating any procedure in these cases the fact that recovery and diuresis tend to occur spontaneously at this time must be remembered

In the present study certain cases that received a water and salt overload early (forcing fluids) had a later onset of diuresis than the cases that received smaller quantities of fluid as in the three phase management Conversely cases with a partial water deficit had the usual onset of the diuresis

Since the forcing of fluids did not initiate diuresis and probably merely added extra leads to the various fluid compartments the type of fluid excess depended upon the composition of the fluid given It is not surprising that added stimuli do not initiate diuresis Since altered plasma composition and acidosis are normally great stimuli to urine formation the kidneys are already under a continuous stimulation *Time for spontaneous recovery seems to be the main requisite*

The peak of diuresis was followed by a drop in urine volume to lower levels A stabilization of the volume soon followed but polyuria continued at a lower level as renal clearance remained depressed A drop that was precipituous indicated a failure to maintain an adequate intake in the face of the diuresis Under such circumstances signs of extracellular dehydration became evident In extreme cases convulsions occurred These complications yielded dramatically to the proper fluid intake The progress of the oliguria-diuresis curve was again used as a clue to the state of the patient

The urine specific gravity remained depressed usually 1 005 to 1 012 throughout both the oliguria and diuresis phases Improvement of this abnormality was slow subsequently

insufficiency has been altered The alterations have been based on two premises (a) the morphologic renal changes in the kidneys and (b) certain major clinical abnormalities The morphologic renal changes indicate that it requires time (8–12 days) for recovery and regeneration of damaged nephrons (figs 7 and 8) The clinical



FIG 7 SECTION OF KIDNEY FROM RABBIT IN EARLY PHASE FOLLOWING AN ACUTE HEMOLYTIC TYPE OF REACTION

Notice a complete disruption (necrosis) of tubules and focal inflammation The cytoplasm of remaining tubule cells show degenerative changes This type of damage requires time for healing and under such circumstances a kidney is in no position to handle large amounts of water and salts nor to be stimulated by various drugs

course offered a natural cleavage into three phases and for this reason the proposed management has been termed the three phase management

The main steps in the three phase management are few in number Anemia or hypovolemia with hypotension during the first phase (reaction shock) are treated with adequate volumes of whole blood At times as much as 2500 cc of blood have been given for this purpose With certain reservations, the clinical picture and the degree of hemodilution may be used as guides to therapy [4, 43]

rhage for which the transfusion was given Other concomitant complications were anemia in two cases severe pneumonitis with cyanosis (hypoxemia) and pyelitis of pregnancy with recent blood loss

There was no significant correlation between the development of renal in sufficiency and the volume intake of incompatible blood, up to 500 cc Within the renal insufficiency group 50 per cent of the cases received 350–1000 cc of in compatible blood and the other half received 60–250 cc In the group without renal insufficiency, however, 37 5 per cent received 400–500 cc and the remainder re ceived 40–250 cc Considering that there were 10 cases of the former and 8 cases of the latter the differences are not obviously as great as one might expect Notable exceptions concerned 2 of the 3 fatal cases who received single incompatible trans fusions of 1000 cc (one under general anesthesia one in coma) These observations which in general agree with Bordley s [7] conclusion that cases receiving larger volumes tend to be more serious, lend additional emphasis to the role of other factors Among these variable factors may be included (a) the relation of antigens to antibodies,[7] (b) the complications at the time of the hemolytic reaction, and (c) the type of therapeutic management to which the patient is subjected

No special relationship was noted between Rh incompatibility and the development of renal insufficiency The groups with and without renal insufficiency each entailed five instances of hemolysis due to Rh antigens

The mortality rate in the present report 17 6 per cent of the cases having a hemolytic reaction is quite low when compared with other series [3] The rate would be lower were we to consider only cases receiving single incompatible transfusions for of the 3 fatal cases 2 had repeated transfusions amounting to 2650 cc and 2500 cc of blood

The results of the present study make one wonder as to how much of the poor past record in the management of these cases has resulted from the rationale used in the treatment A study of reported cases reveals four outstanding faults in management First there is a frequent failure to administer adequate quantities of blood in those cases with anemia or hypovolemia during and after the stage of hemolysis (reaction shock phase) The reports of Hesse and Filatov[41] who considered blood transfusions very beneficial constitute an exception (as found in the literature) to this feature Second there has been a frequent and almost universal attempt to force the kidneys into action during the oliguric period most often by a vigor ous fluid intake In a recent discussion Lattimer[47] has emphasized the fallacy of this practice Third a failure to mention the need for salt replacement during the diuresis phase Fourth the infrequent mention of the electrolyte pattern of the plasma (or serum) in these cases

The frequency of generalized edema in the reported cases has been striking so much so that this complication has been an implied component of the syndrome of acute renal damage We believe that generalized edema should not be a com ponent of the syndrome resulting from incompatible transfusion under proper management but that its occurrence is induced by a fluid intake which surpasses the capacity of damaged kidneys

In the present study the conventional management of cases with acute renal

led to a slight to moderate water deficit after five to eight days No detrimental complications seemed to result from this slight to moderate dehydration during this period (2) The fluid in over 70 per cent of the cases treated with this management was given in the form of a formula* containing 60–125 grams of protein and approximately 1500–1800 calories Although nausea and occasional episodes of vomiting were encountered, most of the formula was tolerated in these cases Mild sedation was of help at this time Excessive nausea and vomiting occurred most often in the overhydrated patient (3) Daily requirement of Vitamins C and B (thiamin niacin riboflavin) were supplemented by the use of water soluble vitamins given intravenously (4) The carbon dioxide combining power of plasma and the serum Na, K and Cl concentrations were frequently noted For Na and K determinations the flame photometer[15] was quite practical Acidosis and hyponatremia were checked by the administration of sodium bicarbonate (per orum or rectum) Attempts were made to maintain the CO combining power of plasma above 50 volumes per cent and the serum Na concentration above 135 mEq/L This feature of the management has been based on the empiric undesirability of acidosis The actual sodium intake by this means has not been great and care has been exercised to prevent a sodium overload Sodium bicarbonate has been given in doses of 6–15 grams in a minimal water content as indicated by the blood chemical analysis No attempt has been made to alkalinize the urine once renal insufficiency was definitely shown

Dangerous hyperpotassemia and hypocalcemia have not been encountered in the hemolytic cases Hyperpotassemia was dangerous in one case with hypotension renal insufficiency and massive blood transfusion

During this technic of management no attempt was made to force a lowering of the nitrogenous waste products during the renal insufficiency phase Mental clarity has been noted with blood urea levels of 250–300 mg per 100 cc and only slight to moderate mental dullness was associated with a level of nearly 450 mg per 100 cc of blood

The main emphasis during the third phase (salt losing diuresis) has been on the replacement of water and salts lost in the urine During this period the main complications (azotemia hypertension, altered electrolyte pattern anorexia) are reversed toward normal but severe dehydration threatens In averting this complication the replacement of water and salts has been made on as near as possible a gram for gram basis by measuring the urine volume and salt output

The main salt excreted is sodium chloride As the eating habits are improved it is not always necessary to supplement the intake of other salts The salt water needs may amount to 20–40 grams and 5 000–10 000 cc daily In our cases this demand has not been prolonged over four to five days With more advanced recovery water salt conservation occurs and the patient may be allowed to regulate his own intake and output The output continues above normal as complete re-

* The approximat composition of th formula has been as follows 1000 calories per liter with the following caloric p rcentages protein 25 per cent fats 25 per cent and carbohydrates 50 per cent The estimated total sodium and potassium content has been 0 5 gram and 0 8 gram respectively At times 2 to 4 eggs have been add d to this formula

Naturally there must be complete confidence in the compatibility of the infused blood When correct blood has not been promptly available in sufficient quantities and hypotension has been severe, we have used plasma solutions as a temporary means of elevating the blood volume At such times concentrated protein solutions



FIG 8 SECTION OF KIDNEY FROM CASE 4 OF HEMOLYTIC REACTION GROUP

This patient had other severe complications as overwhelming pulmonary tuberculosis but lived fifteen days Notice heme casts surrounded by desquamated tubular epithelium The tubules are lined by newly regenerated flattened epithelium In some tubules this epithelium has gained a considerable amount of cytoplasmic substance Therefore at fifteen days regeneration and recovery are well advanced

seem preferable [44] Care must be taken during these emergencies not to overdilute the circulating red cells

The management of the second phase (renal insufficiency) has included four main points (1) No attempt has been made to force damaged kidneys into action On the contrary during the oliguric period the fluid intake has been limited to the estimated insensible fluid loss plus a volume equal to the urinary output As a rule 1000–1800 cc of water per 24 hours were administered for this purpose plus the equivalent of the urine volume At times this was a conservative estimate and

principles and the main clinical abnormalities The outstanding premise for the "three phase" management is based on the consideration that damaged kidneys require time for regeneration and recovery

6 Five main steps in the proposed method of management are (a) adequate compatible blood for anemia and hypovolemia (b) limitation of fluid intake during renal insufficiency to that immediately lost (c) adequate dietary intake, (d) prevention of severe acidosis, (e) adequate water and salts replacement during diuresis

7 Support was gained for this regime by successful application to other types of acute renal damage and insufficiency Demonstrated complications were averted

8 Common complications due to other types of management were anemia and hypotension, fluid overload with cerebral and pulmonary signs, dehydration with shocklike state and at times convulsions during the recovery phase

9 Peritoneal irrigation in its present form resulted in two major complications averted by the proposed method of management

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covery of renal clearance may require weeks to a few months The former condition of the kidneys necessarily influences this terminal phase of convalescence

Six cases of hemolytic transfusion reaction were treated by the three phase type of management Four of these had severe renal insufficiency (Cases 1, 2 6 9) Recovery was rapid and satisfactory in all of these cases Altogether 11 cases of severe acute renal insufficiency of various types have been treated with the three-phase type of management Recovery of renal function within eight to twelve days occurred in each instance Ten cases lived, 1 case died from other complications after recovery of renal function (One case not included in present report)

The three-phase type of management was instituted with success in 2 cases after complications from other forms of therapy had occurred The most common complication consisted of a water or water salt overload (seen in 5 transfusion cases, 3 hypotension cases 1 prostatic resection case and 1 case of CCl_4 poisoning) This complication was characterized by generalized edema (subcutaneous and pulmonary) and cerebral signs The oliguria persisted and in 4 such cases the peak of the diuresis occurred distinctly later (sixteenth–twenty third day) than in the three phase management group Seven of the 10 cases with this complication recovered after a very stormy course The cerebral signs were of the type frequently attributed to uremia but appeared to be due in these instances to fluid overload

The character of the oliguria diuresis curve has not been altered by this fluid overload This is taken as an additional indication that renal regeneration and recovery require a definite time and that neither grave alterations in the composition pattern of the blood plasma nor an excess in quantity of water and salts can force damaged kidneys into proper function before this time has elapsed

On two occasions convulsions occurred during the diuresis phase probably as the result of an insufficient replacement of water and salts Two additional cases displayed signs of advanced dehydration at this time These abnormalities yielded rapidly to the proper water and salt intake

The complications associated with peritoneal irrigation as a means of tiding the patient over the oliguric period have been discussed elsewhere [33 46] In these cases (2 included above) severe acidosis and a water salt overload were associated with the use of various crystalloid solutions as irrigating fluids These are the main complications which the three phase management attempts to avoid

SUMMARY AND CONCLUSIONS

1 Of 28 630 blood transfusions administered in Baylor Hospital over an eight year period there were 17 known hemolytic reactions (0 593 per 1000) of which 17 6 per cent were fatal

2 Acute renal insufficiency occurred in 55 5 per cent of 18 cases of hemolytic reaction

3 Factors other than hemolysis per se often occurred The most common complication was hemorrhage with hypotension

4 The mortality rate of the hemolytic cases with acute renal insufficiency need not be as high as one gathers from reports in the literature

5 Good results may be obtained with a regime based on physiopathological

Rh ANTIBODIES, CORRELATION WITH CLINICAL FINDINGS

By I DAVIDSOHN, M D

THE discovery of so-called incomplete or blocking antibodies and of thermostable agglutinins reacting in serum or albumin but not in saline has stimulated discussion and speculation on their relation to each other and to the previously known Rh agglutinins which react in saline solution and more recently on their relation to the various forms of fetal erythroblastosis

The problem of the nature of the various forms of Rh antibody is one to be solved by immunologic and immuno chemical methods The second problem of the respective role of the antibodies in the genesis of the different forms of the disease may be brought closer to solution by qualitative and quantitative study of antibodies during pregnancy and correlation with the condition of the newborn infant This report aims to present the results of such a comparative investigation

It is based on a study of the titres of Rh antibodies (saline agglutinins serum albumin agglutinins and blocking antibodies) in the blood of 73 mothers of erythroblastotic infants or fetuses In most instances repeated blood samples were obtained at intervals during the pregnancy and after childbirth In some cases tests extended over several years Ten women had only one sample examined but the average number for the remaining 63 women was 4 5 samples

The technic of the various tests was essentially the same as described by Wiener and by Diamond It was presented in detail in a chapter on blood groups in the forthcoming new edition of Kracke and Parker s Clinical Pathology [1]

Various opinions have been expressed regarding the nature of the 3 varieties of Rh antibodies Agglutinins which react in physiologic saline solution are called bivalent antibodies by Wiener The antibody which reacts best in serum or plasma or in a solution of albumin human or bovine is called by him glutinin (formerly conglutinin) Diamond refers to it as heat resistant antibody an appropriate descriptive term Diamond believes that blocking antibodies and antibodies reacting in serum albumin are a manifestation of more intense immunization than agglutinins (hyperimmune antibodies)

It is not within the scope of this paper to take sides in the dispute on the identity or diversity of the various Rh antibodies Factual evidence available does not yet justify definitive statements Reactivity in one or another menstruum, thermal resistance or thermal range, may be manifestations of quantitative and not necessarily of qualitative difference

The following terms will be used here saline agglutinin for the thermolabile antibody reacting in physiologic solution of sodium chloride blocking antibody for the incomplete antibody of Race and Taylor serum albumin agglutinin for the

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ing antibodies and agglutinins are mutually exclusive, at least within the range of certain dilutions. Table 2 is an example of the supposedly simultaneous presence of saline agglutinins and of blocking antibodies in the same serum. Many such records have been reported. In such serums there is agglutination in the undiluted serum, more or less distinct traces in one or two further tubes, perhaps up to dilutions of 1:10 or 1:20 with saline, fairly strong blocking antibodies (f.i. up to a dilution of 1:80) and still stronger agglutination (f.i. 1:2560) in dilutions of the serum with human serum or bovine albumin. Such tests are usually reported as: saline agglutinins 1:10, blocking antibodies 1:80, serum albumin agglutinins 1:2560. Such interpretation and reading of the result is not justified, because what is interpreted as saline agglutinin in this and similar cases is not the usual saline agglutinin, but a serum albumin agglutinin strong enough to react weakly in low

TABLE 2

Dilution of Serum	Saline Agglutinin	Blocking Antibody	Serum Albumin Agglutinin
Undiluted	+	Present	+
1:5	+w	Present	+
1:10	±	Present	+
1:20	−	Present	+
1:40	−	Present	+
1:80	−	Present	+
1:160	−	Absent	+
1:320	−	Absent	+
1:2560	−	Absent	+
1:5120	−	Absent	−

dilutions of saline. In this report such serums are listed as lacking saline agglutinins.

As shown in table 3, in 28 cases (38 per cent) blocking antibodies were found. Twenty mothers gave birth to stillbirths or to infants with hydrops dying within a few hours after birth. Eighty five per cent of these mothers had, and only 15 per cent lacked, blocking antibodies. In the remaining 27 deaths blocking antibodies were present only in 7 per cent of the mothers, while in the majority (93 per cent) of postpartum deaths not due to hydrops blocking antibodies were absent. They were present in only 18 per cent of the 50 mothers who gave birth to babies with icterus gravis. In the mothers of 26 survivors blocking antibodies were present in 35 per cent.

The incidence of the 2 groups of antibodies was approximately equal in the total numbers of cases, deaths and survivals. Impressive differences are noticeable in mothers of stillbirths, of hydropic babies and in those receiving transfusions, where the blocking antibodies were higher, and in mothers of babies born alive and free of hydrops, and of babies with icterus gravis where the titre of blocking antibodies was low.

The relatively low figure for sera with agglutinins (62 per cent) is at variance

so-called conglutinin or glutinin of Wiener* globulin antibody for the red cell coating globulin which reacts with the anti human globulin rabbit immune serum of the Coombs test (Hill's developing test) The terms are admittedly cumbersome but descriptive by inclusion of a prominent feature of the tests used for their detection Some terms have been discarded because they are based on unproved hypothetical concepts It is hoped that more fitting and less awkward terms will be substituted as soon as the true nature of the antibodies will become known Table 1 presents the tests used for detection of the various forms of the Rh antibody

TABLE 1

Tests for Rh antibody				Forms of Rh antibody			
				1	2	3	4
Serum tested	Diluent	[illegible]	Clumping	Thermolabile Rh agl (saline agg)	Incomplete or blocking antibody	Thermostable Rh aggl (reacting in serum or albumin)	Globulin antibody (Coombs test, Hill's developing test)
Known anti Rh (control)	Saline	Rh — red cells	Yes	*present*			
1 Unknown		"	Yes	*present*			
2 Unknown			No	absent			
2a Unknown			No plus known anti Rh serum—clumping	absent			
2b Unknown			No plus known anti Rh serum—no clumping	absent	*present*		
2c Unknown	"		No centrifuge remove saline add human serum or bovine albumin—clumping			*present*	
2d Unknown			No centrifuge remove saline add anti human globulin rabbit immune serum—clumping				*present*

Thermolabile Rh agglutinin and blocking antibody are never present together
Thermostable Rh agglutinin may be present together with the thermolabile Rh agglutinin or with the blocking antibody

The material to be presented was divided into 2 main groups (1) Cases with blocking antibodies and serum albumin agglutinins but without saline agglutinins (2) Cases with saline agglutinins and serum albumin agglutinins but without blocking antibodies

This division was suggested by the observation that in our material such combination was the one usually encountered It is in accord with the fact that block

* It is possible that it is not the presence or absence of sodium chlorid which is responsible for the differences in reactivity of saline agglutinins and serum-albumin agglutinins but the water content of the diluent In such a case the term hydrophilic may be the appropriate adjective for the saline agglutinin and hydrophobic for the serum-albumin agglutinin

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tinins and serum albumin agglutinins (but no blocking antibodies) had stillborn babies and 91 per cent had babies with icterus gravis The incidence of hemolytic anemia as the presenting manifestation (without jaundice) was too small to permit evaluation The small number of cases of fetal erythroblastosis with anemia as a predominant feature is probably also explained by the referral mostly of severely affected children as patients to the hospital and of their mothers for serologic study in the laboratory

Wiener suggested recently that agglutinins (saline agglutinins) are mainly responsible for icterus gravis, and the so-called conglutinins (serum albumin agglutinins) and blockers (blocking antibodies) or, as he calls them univalent antibodies are mainly responsible for the severe damage leading to intrauterine death and hydrops [3] In his series agglutinins alone (saline agglutinins) were found in 64 per cent of mothers whose babies had icterus gravis whereas blockers and conglutinins were present only in 14 per cent of such mothers The mothers of stillborn babies were found to have agglutinins in only 8 per cent and conglutinins and blockers in 43 9 per cent of cases

TABLE 5 —*Correlation of Saline Agglutinin/Serum Albumin Agglutinin Ratio with Survival*

Ratio of saline agglutinin serum albumin agglutinin titers		Died		Survived	
	No	No	%	No	No
Titre of saline agglutinins higher than or equal that of serum/albumin agglutinins	22	10	45	12	55
Titre of serum albumin agglutinins significantly higher than that of saline agglutinins	23	18	78	5	22

The serious import of blocking antibodies is apparent from Wiener s and our results On the other hand agglutinins seem to have a less serious implication In table 5 the 45 cases with serum albumin agglutinins and saline agglutinins but without blocking antibodies were divided in 2 groups one in which the titre of saline agglutinins was higher than or equal to that of serum albumin agglutinins (allowing one tube difference for technical factors) and the other group in which the serum albumin agglutinin titre was significantly higher than the saline agglutinin The lower death rate in the first group is impressive though the sample may be too small to be significant

Anamnestic Rh antibody reaction It is a well known fact that nonspecific stimuli may cause a reappearance of antibodies that had in the past been produced by a specific antigen and had disappeared from the circulation in the course of time This is the so called anamnestic reaction also known as the Hektoen phenomenon The dictionary defines it as follows [4] When antigens are introduced into the animal body in allergic states there may exist an increased range of new antibody production which may include production of antibodies concerned in previous infections and immunizations

We had an opportunity to observe what may be interpreted as anamnestic Rh

with my previous report of an 82 per cent incidence [2] This is explained by the inclusion previously of serums with agglutinins which are listed as free of saline agglutinins in this paper and as containing blocking antibodies and serum albumin agglutinins only Tests for blocking antibodies and serum albumin agglutinins had not been used in the study previously reported

The high mortality in this series is due to some extent to the fact that some babies were sent in from other hospitals in critical condition making this a select group of cases

Table 3 —*Distribution of Rh Antibodies*

	Blocking Antibodies and Serum Albumin Agglutinins but no Saline Agglutinins			Serum Albumin Agglutinins and Saline Agglutinins but no Blocking Antibodies	
	No	No	Per cent	No	Per cent
Total cases	73	28	38	45	62
Deaths	47	19	40	28	60
Stillbirths and hydrops	20	17	85	3	15
Deaths without stillbirth and hydrops	27	2	7	25	93
Icterus gravis	50	9	18	41	82
Survivals	26	9	35	17	65
Mothers who received transfusion prior to birth of affected child	12	11	92	1*	8

Mother of stillbirth

Table 4 —*Correlation between Rh Antibodies in the Maternal Serum and the Clinical Manifestations in the Infant or Fetus*

Type of Antibody	No of Families	Stillbirth & hydrops			Icterus Gravis & Nuclear Jaundice			Hemolytic Anemia		
		No	%	±P E	No	%	±P E	No	%	±P E
Blocking antibodies and serum albumin agglutinins but no saline agglutinins	28	17	60 7	±6 0	9	32 2	±5 7	2	7 1	±3 3
Saline agglutinins and serum albumin agglutinins but no blocking antibodies	45	3	6 7	±2 6	41	91 1	±2 7	1	2 2	±1 4

Twelve mothers had a history of transfusion prior to the pregnancy under consideration The number is small but it may be significant that 11 or 92 per cent had blocking antibodies only 1 mother had none and the baby of this mother was stillborn Six of the transfused mothers (55 per cent) had stillborn babies

In table 4 the antibodies are correlated with clinical manifestations in the newborn Sixty per cent of mothers with blocking antibodies had stillborn or hydropic babies 32 per cent had babies with icterus gravis and 7 per cent had babies in whom hemolytic anemia was the predominant finding and jaundice was not striking On the other hand only 6 7 per cent of mothers with saline agglu

tinins and serum albumin agglutinins (but no blocking antibodies) had stillborn babies and 91 per cent had babies with icterus gravis The incidence of hemolytic anemia as the presenting manifestation (without jaundice) was too small to permit evaluation The small number of cases of fetal erythroblastosis with anemia as a predominant feature is probably also explained by the referral mostly of severely affected children as patients to the hospital, and of their mothers for serologic study in the laboratory

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We had an opportunity to observe what may be interpreted as anamnestic Rh

antibody reaction in case 6 The obstetrical history, the blood groups, the Rh factors of the family and the levels of Rh antibodies before and during pregnancy are recorded in figure 1 Anti Rh agglutinins of a low titre (1 8) were found in 1943, 5 days after delivery of a stillbirth, with typical findings of fetal erythroblastosis (hydrops) Tests for serum albumin agglutinins and blocking antibodies were not done After artificial insemination (donor A, Rh negative MN) the woman became pregnant in November 1945 No Rh antibodies of any kind were found at approximately the end of the third month of pregnancy, January 31 1946 Seven specimens

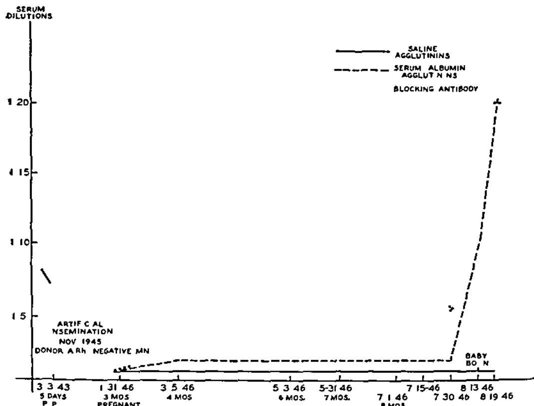


FIG 1 ANAMNESTIC Rh ANTIBODY REACTION No 6

Father A Rh positive Mother AB Rh negative Pregnancies (1) 1936 ♀ Stillbirth 6 months weight 705 Gm (2) 1936 ♂ Normal A Rh positive (3) 1943 (Feb 26) Term ♀ Stillbirth hydrops Weight 2800 Gm Placenta 1620 Gm F E (4) 1946 (Aug 13) Term ♀ Normal B Rh negative

were examined at intervals varying from 2 weeks to 2 months At the beginning of the fourth month saline agglutinins could not be found but traces of serum albumin agglutinins and blocking antibodies were observed for the first time Serum albumin agglutinins were very weak detectable only in undiluted serum until the day of delivery when the titre rose to 1 10 Blocking antibodies reached their peak (1 20) in the seventh month and remained at that level except for a brief drop 2 weeks prior to delivery Both antibodies were still present one week after delivery

The appearance of the antibodies early in the pregnancy caused concern in view of the past obstetrical history It was thought possible that the pregnancy resulted

not from the artificial insemination, though the patient and her husband asserted that they had adhered strictly to instructions and that the husband could not possibly be the father The delivery of a normal B Rh negative child showed that the antibody response could not have been due to a specific stimulus by the Rh factor It is known that in heterospecific pregnancy the factors A and B may act not only as specific but also as nonspecific antigenic stimuli For instance, in women of group O, a rise of anti A agglutinins was observed in the course of pregnancy with a baby of group A In the following pregnancy with a baby of group B there was not only a rise of anti B but also of anti A iso-agglutinins A rise of both anti A and anti B iso-agglutinins was also observed in pregnancies where the infant was found to be of group O or of the same group as the mother In case 6 the presence of factor A in the donor of the semen and in the mother made him an unlikely source of antigenic stimulation, especially since the baby did not inherit the A factor The mother was not tested for factors M and N If she lacked one of them then it may possibly be incriminated though factors M and N are extremely rarely antigenic in man However there is little doubt that our knowledge of individual antigenic differences is still too limited to exclude the presence of a hitherto unknown antigen in the fetus and its absence in the mother According to present knowledge such an antigen would be nonspecific with regard to the Rh factor, thus justifying the reference to the observed phenomenon as anamnestic Rh antibody reaction

The persistence of antibodies The persistence of Rh antibodies in the circulation varies from case to case depending on the intensity and duration of immunization the potency of the antigenic stimulus the individual response and, in the case of immunization during pregnancy, on the number of pregnancies and on the permeability of the placenta The role of transfusions prior to pregnancy has already been discussed Case 10 is an example of persistence of Rh antibodies for at least 36 months and probably for over 4 years Twenty seven samples of blood were examined at regular intervals during the last 3 years The titres of saline agglutinins and serum albumin agglutinins are recorded in figure 2 Blocking antibodies have been absent consistently

Value of repeated tests Repeated examination of the blood of pregnant women for antibodies offers a unique opportunity for the study of responses to antigenic stimuli The emphasis must be placed on the words repeated examination because a single examination may be misleading partly due to variations in the agglutinability of red cells even if the cells of the same person are used for all tests Another reason is the possibility of persistence of antibodies from previous pregnancies The examination should begin preferably not later than the third month of pregnancy Repeated tests (at from 1 to 2 month intervals and at biweekly intervals after the seventh month) make it possible to recognize a trend in antibody production which may permit clinical interpretation

Case 34 Woman age 29 gravida 2 para 1 A Rh negative received 2 transfusions when ill with osteomyelitis at 15 The husband was A Rh positive First child boy O Rh positive was born 5 years prior to this examination He had icterus gravis and anemia received 3 transfusions and recovered fully Anti Rh

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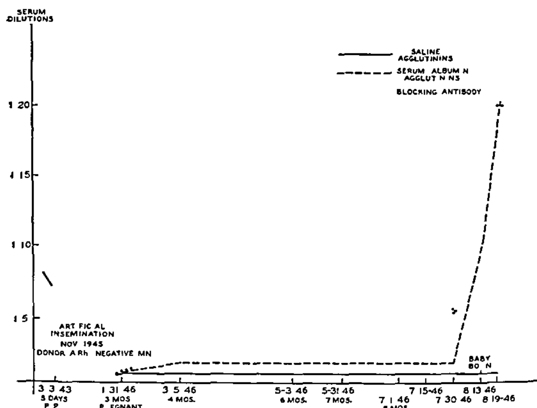


Fig 1 Anamnestic Rh Antibody Reaction No 6

Father A Rh positive Mother AB Rh negative Pregnancies (1) 1936 ♀ Stillbirth 6 months weight 705 Gm (2) 1936 ♂ Normal A Rh positive (3) 1943 (Feb 26) Term ♀ Stillbirth hydrops Weight 2800 Gm Placenta 1620 Gm F E (4) 1946 (Aug 13) Term ♀ Normal B Rh negative

were examined at intervals varying from 2 weeks to 2 months At the beginning of the fourth month saline agglutinins could not be found but traces of serum albumin agglutinins and blocking antibodies were observed for the first time Serum albumin agglutinins were very weak detectable only in undiluted serum until the day of delivery, when the titre rose to 1 10 Blocking antibodies reached their peak (1 20) in the seventh month and remained at that level except for a brief drop 2 weeks prior to delivery Both antibodies were still present one week after delivery

The appearance of the antibodies early in the pregnancy caused concern in view of the past obstetrical history It was thought possible that the pregnancy resulted

found to clump cells of group O At that time routine tests for the Rh factor had not been done The irregularity was explained later by finding that the woman

TABLE 6 —*Drop of Antibodies in Maternal Serum Preceding Stillbirth*

Case No 35*

	Saline Agglutinins	Serum albumin Agglutinins	Blocking Antibodies
January 29 1945 Pregnant 8 months due February 28	40	—	0
February 6	1	—	80
February 26 Birth of stillborn hydrops Path Typical Erythroblastosis			
March 6	1	40	10
April 11	1	40	10
November 26	1	80	5

* Father—A Rh_0 Rh_0 Hr positive
Mother—A Rh negative anti Rh_0
Pregnancies 1 ♂ A Rh positive 3 years normal
2 ♂ Stillborn February 26 1945

TABLE 7 —*Value of Periodic Tests for Antibodies*

Weak antibodies during last weeks of pregnancy
Normal child
Case 31 Father ORh+Hr+
Mother ORh−
Pregnancies
1 ♀ 1938 ORh− 5½ years normal
2 1939 Abortion 2 months
2 transfusions from husband
3 1941 Abortion 7 weeks
4 1943 Abortion 4 months

High titer of antibodies throughout pregnancy Severe icterus gravis

Case 30 A Father ORh_1Hr-
Mother BRh− anti Rh_0
Pregnancies
1 ♀ ORh+3 years normal
2 ♀ BRh+ F E I G died 3 days

5 months pregnant	Saline aggl	Blocking antibodies	3 months pregnant	Saline aggl	Serum albumin aggl	Blocking antibodies
7/11/44	0		4/25	80	320	0
9/15/44	0		6/5	80	80	0
10/12/44	0		7/15	40	20	0
11/17/44	+(1)		8/22	160	320	0
12/ 4/44	Normal ♂ ORh+		9/24	160	1280	0
			♂ BRh+ born 10/12/46 Severe icterus gravis recovered			
12/ 7/44	+(1)*					
12/16/44	+(1)*	+	10/14	2560	5120	0
			10/18	1280	2560	0

* Clumping only in undiluted serum

was Rh negative and had anti Rh agglutinins (titre 1 40) Two days later she reported to her obstetrician that she did not feel movements of the baby Blood examined on February 6 showed agglutinins present only in undiluted serum and a

agglutinins of low titre (only in undiluted serum) were found in mother s blood a few days after the delivery, and again 3 years later (December, 1944) Patient returned in September 1945 to be tested for Rh antibodies She was then 3 months pregnant The titre of agglutinins was still at the same level as in December 1941 Six weeks later (October 22) the titre of agglutinins was unchanged but the titre of *serum albumin agglutinins had risen to 1 160* On December 10 the titre of serum albumin agglutinins dropped to a level of 1 5 and persisted at this level until the end of the pregnancy and beyond it There was a trace of blocking antibodies (only

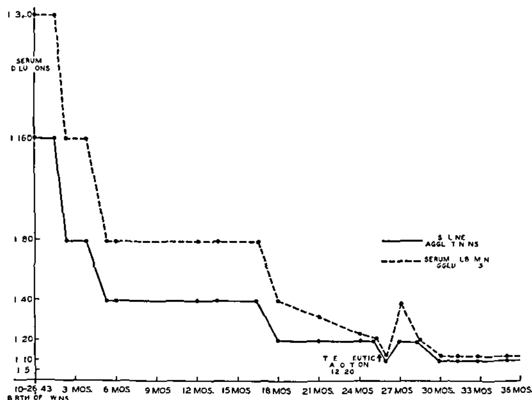


FIG 2 PERSISTENCE OF Rh AGGLUTININS AND CONGLUTININS OVER THREE YEARS

Father O Rh positive Hr negative Mother O Rh negative anti Rh_0 agglutinins Pregnancies (1) 1941 abortion 3 months (2) 1942 (Aug 30) Term ♂ Icterus gravis Purpura Lived 4 days (3) 1943 (Oct 26) Term ♀ Twins O Rh+ ♂ O Rh+ Icterus gravis Purpura Lived 3 days (4) 1945 (Dec) Therapeutic abortion 5 weeks

in undiluted serum) On February 6 1946 2 months after the precipitous drop of serum albumin glutinins was noted the patient gave birth to a hydropic stillborn fetus

Was there any relation between the drop of antibodies and the changes in the fetus which led eventually to its death? The appearance of the fetus indicated that it had been dead in utero only about 7 days It may be that the process which eventually destroyed the fetus may have started several weeks before delivery and that the drop of the titre of antibodies may have been due to some such changes

One more example of similar nature

Case 35 During a routine prenatal blood grouping test the patient s serum was

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DISCUSSION

Dr Levine I enjoyed hearing these two very interesting papers A number of points in Dr Diamond s paper could have been anticipated on the basis of what we have known of isoimmunization by pregnancy and what the immunologists know about minute quantities of blood and their action as immunizing agents With reference to Dr Davidsohn s comments on terminology I believe it is no secret that the suitable authorities in Washington are trying to lay down the standards for diagnostic anti Rh serum Some of us have already seen the preliminary draft I am quite pleased with Dr Davidsohn s terms hydrophilic and hydrophobic antibodies for the so-called blocking tests and direct agglutination We have seen a few cases where women had blocking antibodies at a moderate titre and I was of course disturbed as to the outcome in the infant However I was very much surprised to find that the infant was Rh positive and perfectly normal The fact that the infants are Rh positive may indicate that perhaps the blocking antibodies did not go through the placenta or perhaps the antibodies did go through the placenta and the titre was not high enough and there wasn t sufficiently long intrauterine action to affect the cells

Now these latter cases with blocking antibodies are in contrast to 3 cases we observed very early in in our course of study in 1940 and 1941 when we found women with agglutinins and infants who were perfectly normal At that time I suggested that we would never get erythroblastosis until we had prolonged intrauterine blood destruction At the same time it is very important from the public health stand point to detect those mothers who are already immunized in that particular pregnancy

We can be certain that in subsequent pregnancies the immunization will be severe enough for the mothers to have an affected baby and since that will be the first affected baby I am not quite certain whether recommending longer intervals between pregnancies will be effective It will be one of the only things we can suggest at this time to prevent severe reactions So far as the nonspecific increases in agglutinins is concerned years ago when Dr Landsteiner and I were studying production of anti M and anti N sera we occasionally found curiously enough a very weak anti N response when we injected M I can also confirm Dr Davidsohn s remarks that we found in pregnancies nonspecific increases in the anti A and anti B agglutinins when the affected infants were in group O Now in regard to Dr Diamond s paper which I have been looking forward to hearing esp cially his comm nts on the siz of the 2 anti bodies perhaps he can tell us his impression of their siz s and possibly associate this with th p rm a bility of the placenta of these 2 antibodies In our early studies of the erythroblastosis of th first born we made the guess that these women may have been immunized by previous transfusion or by intra muscular injection of whole blood early in life In a few cases we were able to demonstrate that the an amnestic reaction could persist for a long time between the intramuscular injection and th pregnancy We also found evidence which confirms the findings that the number of transfusions prior to the first pregnancy did not influence the severity of the condition in the first infant and I believe that it is cor rect to assume that a single transfusion is equivalent to multiple pregnancies

Soon after we described the pathogenesis of erythroblastosis I was puzzled as to the m chanism of the immunization I didn t commit myself for a period of a few years and in the meantim low Vitamin B was suggested before we demonstrated intraplacental hematomas with fetal blood on the maternal side

* Dr L K Diamond s paper Physicochemical and Immunological Characteristics of Rh Anti bodies presented at the Conference was planned for appearance in this issue but unfortunately was not received in time for inclusion *The Editors*

high titre of blocking antibodies (1 80) The same day a stillborn hydrops was delivered (table 6) This case poses the same question as the preceding The relation between the drop of the titre and the stillbirth is even more suggestive The impression is supported by the usual striking persistence of titres during pregnancy with only slight variations readily explained by technical factors and by fluctuations in the sensitivity of the Rh positive test cells

The value of periodic tests for Rh antibodies is also shown in the 2 cases recorded in table 7 In case 31 the mother had a bad obstetric history with 3 abortions and 2 transfusions from the Rh positive husband Repeated tests were negative till 17 days before delivery when for the first time agglutinins were seen, but only in undiluted serum Three and 12 days after delivery of a normal Rh positive child the titre was the same In addition blocking antibodies were detected for the first time at the last examination (done later on a preserved frozen specimen) It is possible that accumulated experience may permit us to draw favorable conclusions from such a course

For comparison case 30A is recorded in the same table to show an entirely different course of antibodies in a pregnancy terminating in the birth of a baby with severe icterus gravis The findings in the last 2 cases are in accord with the recent report by Page Hunt and Lucia[5] of a direct relationship between the duration of exposure to maternal antibodies and the occurrence and severity of erythroblastosis in the infant

A series of patients in whom tests for Rh antibodies were done repeatedly during pregnancy was studied recently from the same point of view as presented in the paper of Page and associates and will be reported [6]

SUMMARY

Rh antibodies were studied during pregnancy at frequent intervals in the blood of 73 mothers of babies with fetal erythroblastosis The serums containing antibodies were divided in 2 groups (1) serums with blocking antibodies without saline agglutinins (2) serums with saline agglutinins and serum albumin agglutinins but without blocking antibodies The correlation of these 2 groups with clinical findings in the newborn showed striking differences blocking antibodies present in 85 per cent of mothers of babies with hydrops or stillborn and only in 9 per cent of mothers of babies with icterus gravis

Predominance of saline agglutinins over serum albumin favored survival and vice versa

In a small group of women with a history of transfusions who gave birth to erythroblastotic babies blocking antibodies were found in 92 per cent

The number of cases used for this study is admittedly small and only very guarded conclusions may be drawn The results however are in general agreement with recent studies of Wiener on correlation of antibodies with clinical manifestations and with results of Levine and others regarding the sensitizing influence of blood transfusions

Periodic tests for antibodies during pregnancy may permit prognostic conclusions

An anamnestic Rh antibody reaction is described

Dr Potter I've been extremely interested in the difference in the number of patients in whom agglutinins have been determined in the English group and the American group I think Boorman in that group reported agglutinins in 93 out of 97 Rh negative women and I've been curious as to whether they are using some more sensitive technic which might have included blocking antibodies as an explanation of the difference between the percentage found by the English and American investigators

Dr Race The only explanation I could offer is that they must have been reporting the presence of very small traces of agglutinins This was done before the blocking or incomplete antibody was described They found these only in their first tubes and they were really doing the so-called conglutinations

Dr Levine The tests on the mother's serum alone is not a very good criterion of the outcome of a pregnancy It is very important to test the cord blood to determine the titre in the infant's blood at delivery and to follow the titre in order to obtain a true concept of the prognosis In the slide I showed yesterday 2 contrasting cases sent to me by Dr Stillman of the New York Hospital are shown In both cases the mother had positive albumin tests with a titre of 1 512 In one of them the infant had practically all of the mother's antibodies and it could have been expected that the baby would not survive In the other one there is only a blocking antibody of 1 4 and a good prognosis was anticipated and the infant recovered with only 1 transfusion I would like to find out the experience of the other workers here with regard to following up the infant It is difficult to obtain specimens of blood from infants and developing tests could be done on a suspension of the infant's blood cells without obtaining serum I would like to have the comments of Dr Race or Drs Hill and Haberman on this point

Dr Hill I want to thank Dr Diamond and Dr Davidsohn for 2 very interesting papers we're all so tremendously interested in these antibodies and their nature I think that there is one thing I would like to make perfectly clear concerning the developing test We used this term for convenience and we claim no credit or responsibility whatsoever for the development of this test It's distinctly a test of Coombs Mourant and Race The only responsibility or perhaps the word I should use is culpability is in the use of this test to characterize third order antibodies (cryptagglutinoids) if such exist We rather think that there are some antibodies still which are detected by this Coombs test and which are not detected by the albumin test I believe however further work must be done along this line We have seen some such examples and Dr Race tells me of some examples over in England

In response to Dr Levine's question on the developing test and the progress of the affected infant I can state that we have found this test invaluable By daily or semiweekly tests on the child's erythrocytes we have been able to follow the progress of recovery As the antibody and the adsorbed infant cells are disposed the developing test becomes weaker until finally it is negative when the child has completely recovered In one case the test remained positive for one month The clinical condition improved as the test became progressively negative

I would like to mention one thing in connection with the method of injection of the Rh positive cells that Dr Diamond mentioned We have been able to give the 5 cc quite successfully to people with titres of even 1/50 000 to 1/100 000 *with minimal or no reactions* This was *reported in July 1945 in* the Journal of the American Medical Association It's true that some slight reactions have occurred a little headache or discomfort but the more severe reactions have not occurred even in those cases where we did not give a diluted blood We have usually diluted the 5 cc with an additional 5 cc of saline citrate taking care to use at least 10 minutes time in giving it On one or two occasions we got a little curious and gave it more rapidly particularly to some members of our own staff who happened to be Rh negative and were receiving these injections and it seemed to give some more difficulty

In connection with this discussion of antibodies I think it might not be inappropriate to bring up the question of method of typing for Rh Dr Chown who will speak next has a beautiful little test that we are very enthusiastic about for several reasons It is the capillary test We like it because it's simple requires very little equipment and is both rapid and specific The beauty of it is that if you have a potent serum so that you can use it saline diluted to avoid false positive reactions you can utilize the third order antibodies (cryptagglutinoids) To that extent it is similar in its action to the albumin test We are using it routinely and its specificity seems to be the same as the test tube method Its sensitivity is very excellent For the smaller laboratory this is a beautiful test

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of the placenta I suggested that isoimmunization begins at the latter part of pregnancy wh n there are certain structural changes in the villi and the position of the fetal blood vessels would suggest that they are separated from the maternal by only one layer of cells We had calculated that it would require on the basis of some experim ntal studies in rabbits only about 0 07cc of blood in the cours of the entire pregnancy to immunize the mother On the basis of this I am pleased to not that Dr Diamond and Drs Hill and Haberman found that minute amounts of blood will by intravenous inj ction suffice to enhance *the immunization in an already immunized woman I believe that pregnancy offers very suitable con* ditions for immunization giving p rhaps slow administration of an antig n over a long p riod of tim I would like to see someone attempt to immunize m mbers of a family to d termin what at pres nt we *are only guessing at that is to explain the genetic capacity and the variations among individuals to re* spond to antigenic stimulus with the production of antibodies If we could s lect individuals of a family and immunize the siblings of the 2 contrasting groups those that immunize readily and those that do not we could make a notable contribution to the problem of immunization by demonstrating th actuality of such an inherited characteristic Anyone having such an opportunity could not only h lp produc the rare sera but could also establish or disprove the inheritance of the respons to the Rh antigenic stim ulus If this can be done and result in the production of the rare anti Hr s ra in suffici nt quantity th n the proper genetic studies among various races will determine the inheritance involved in the Rh Hr sys tem Until we have this we will flounder and be uncertain about our terminology and will probably hav to use several terminologies

Dr Dameshek I find it difficult to present a direct discussion of the pap rs of Drs Diamond and David sohn in that they deal mostly with terminology as applied to th Rh factor How ver it would b wis to review some of the historical features and broader aspects of the problem As I pointed out yest rday we have gone into this problem from a more basic standpoint b cause erythroblastosis f talis h molytic disease of the new born is just one type of h molytic anemia Th re are oth r typ s of h molyti anemia which should not be forgotten even though this conference d als larg ly with the Rh factor The specialty of immuno-hematology was first introduced by Chauffard a very emin nt French clinician who published and studied hemolytic anemia during the p riod b tween 1905 and 1916 Chauffard was the first to describe the pres nce of hemolysins of a p culiar typ in cas s of hemolytic an mia following th reports of Landsteiner and h described cases of what he called hemolysinic an mia Simultan ously with Chauffard Widal Abrami and Brulle describ d cas s of h molytic an mia in which th y found autoagglutins As a result an active rivalry existed in the French school b tw en th adh rants of Chauf fard and the adherants of Widal Abrami and Brulle as to who would d scrib which typ of cas Chauf fard at that time introduced a concept of immuno-h matology and suggested that th re might som day develop a sp cialty in this particular fi ld That idea lay dormant for many years until th rec nt de velopments especially the Rh factor This sp cialty is now coming to the fore esp cially as advanced by this conference The developments which have taken place with reference to the Rh factor can b brought over directly to the subject of hemolytic anemias in general as I pointed out yesterday Dr Diamond s development of the bovine albumin technic has been utiliz d by us successfully for th determination of p culiar agglutinins and antibodies in various typ s of hemolytic anemia In 1937 when we first en countered these p culiar cases of hemolytic anemia Dr Schwartz and I discovered these p culiar h moly sins and agglutinins in various typ s of hemolytic anemia This was prior to the developm nts in the field of Rh antibodies and their determinations and when Dr Levine introduced his theory that erythro blastosis fetalis was due to an antibody antigen combination it was easy for us to understand that particu lar mechanism because we had been working along similar lines with the hemolytic an mias I would like to point out that we shouldn t los track of the fact that antibodies have reference not only to the Rh factor *and hemolytic anemia but also to hemolytic anemia in gen ral*

Dr Race I have only congratulations to offer to Dr Davidsohn and Dr Diamond for their excellent presentations The scope of their studies is on a larger scale than the work in England

I am *very grateful to Dr Diamond for the extent of his technic for stimulating the antibodies already* present This proved very invaluable to us in getting enough of the 2 rare sera the anti c and the anti Cw

In the case of anti-Cw the large amount of serum obtained was the result of restimulation of the immunized patient I have never before appreciated the very high incidence of immunization in Rh negative men who have been transfused In England our incidence of th detection of antibodies is approximately 2 per cent

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value as with the saline The only time Dr Wiener has used the term conglutinin for a serum is when it contains either no saline agglutinin and positive agglutination with his plasma or serum or when it contains a higher titre of agglutination with the plasma or serum that it do s with the saline So that with these charts which Dr Davidsohn presented if we adhered strictly to the Wiener nomenclature we would have to use the term saline or simply agglutinins for the cases in which th re was no difference between saline agglutination and albumin agglutination and reserve the term conglutinin for the cases where there was a difference either complete or partial As to Dr Levine s comments he has always guarded and supported us and I am delighted that he approved of our work in the sense of having no harsh criticisms since that was my one fear in presenting some of these observations in a field in which we are complete novices and he and Dr Race and others are truly the experts As to the fact of the albumin antibody I suggested that Dr Onkley and his group had found or at least had preliminary observations (and we hope that they will carry these further) and the fact that the albumin agglutinin tends to be a heavier molecule Now whether or not this means that it cannot be a univalent antibody is again beyond my experience or knowledge and immunologists like Dr Heidelberger and Dr Cabot of New York may possibly eventually decide this important theory Dr Cabot has been a great help in many of the dis cussions We have presented him with some of the material and I hope in due time he will be able to give us the answer as to whether the pure albumin antibodies are multivalent or univalent and as to their true chemical structure and particularly their weight I mentioned that we have at least 4 wom n who have received multiple transfusions of Rh positive blood and though they were Rh negative and had pregnancies following it showed no antibody reaction at any time and had no difficulty with their infants In only 1 case were we able to try any tests on a member of that family this case being a woman whose father had been the donor of blood for her transfusion He was the only other living member of her family and he came in and subjected himself to injection with Rh positive blood against which he developed no antibodies again suggesting as Dr Levine mentioned that this may be a familial char acteristic that some humans are just poor guinea pigs in the sense of antibody producers and with a relatively poor antigen such as the Rh factor do not respond by antibody production So that despite the real danger we all must realize that some humans will not develop antibodies even though subjected to the same insults as others by the injection of Rh positive blood Dr Dameshek mentioned that this opens up a much wider field of study particularly in hemolytic anemia We have had that brought to our attention and I think Dr Witebsky will substantiate this by the fact that many of our allergists are now reviewing their work on antibody production and antibody protection in allergic patients who are sensitive to ordinary antigens better known and better studied like egg albumin and rag weed pollens and so on These investigators have assured us that they find exactly comparable states in such individuals in that the heat stabile antibody develops at the multiple sensitization In fact the whole theory of desensitization of allergic individuals by repeated injections of very minute doses of the pollen or the antigen to which they are sensitive is probably dependent not on desensitization but on the developm nt of a more complete antibody which does not permit the symptoms of allergy like hay fever or asthma but protects the individual from such symptoms after a long series of injections Lovel ss in 1941 in Ne York worked on the subj ct and Dr Ableson I think was the first to bring it to my attention Dr Loveless published a beautiful series of papers on heat stable antibodies produced against one of the common antigens after multiple injections which wiped out the skin sensitivity in sensitive individuals who had previously been tested with the antigen and reacted violently with weals The whole field of allergy immunology and certainly hemolytic disease will bear further study and restudy in the light of some of the newer technics

Dr Scherer questioned the use of donors who had received transfusions and might not have been recognized as carrying antibodies because they were not tested by the more sensitive Coombs test or albumin test We have only one very good example of this in that a very patriotic woman who had given blood many times to the Red Cross came to see us with the story that she was interested in our work because she had lost several babies with erythroblastosis This was the beginning of our albumin titra tion technic and we demonstrated that she had a titre of 1 500 of albumin agglutinins She had given blood at least 4 times to the Red Cross her plasma having been used in various pools and at this time we mixed 100 cc of her serum with 20 times that amount of normal plasma and then injected a small sample of 50 cc of the mixture into an Rh positive individual and demonstrated that there was some destruction of the recipient s cells even from this small amount because she had such a high titre Cer

tainly the use of an individual such as she as a donor for an Rh positive recipient would have been extremely dangerous and we therefore should add to our blood bank technic the question of the possible donors having signs of sensitization either from previous transfusions or from previous pregnancies I am sure Dr Hill and others who are responsible for blood banks probably do this because occasionally a donor of that type would truly be dangerous if by mistake he or she were used for an Rh positive recipient Of course in most instances such an Rh negative donor would be used for an Rh negative recipient and therefore no harm would be done But if the plasma were pooled as it was in the Red Cross campaign possibly that might explain some of the untoward effects from giving plasma that were thought to contain pyrogens or nonspecific disturbing antibody We routinely test the cord blood of every new born baby when we suspect sensitization and test it by 3 methods immediately That is the Rh typing is done immediately to determine whether the child is Rh positive or Rh negative and we found both Dr Chown s very simple and effective test or our slide test is adequate for determining within 10 minutes whether the new born baby is Rh positive or Rh negative If the baby is positive and we do not have the mother s blood available for checking we also test the baby s serum to determine whether there are antibodies carried over from the mother Finally we also apply the reagglutination test of Dr Ableson to demonstrate whether the baby has antibody adsorbed red cells which will reagglutinate other Rh positive cells when the two are mixed Incidentally that is a good demonstration of the danger of using Rh positive blood for the transfusion of the babies with erythroblastosis especially during the first 2 days of life If the baby s cells are enveloped or coated with the mother s antibodies and then more Rh positive blood is put into the baby s circulation not only will that positive blood be destroyed more rapidly but the baby s own coated cells will be completely agglutinated in most instances I think this in vitro as well as in a few cases we have studied in vivo mechanisms is the reason for avoiding Rh positive blood in children with erythroblastosis certainly during these first 10 days or 2 weeks of life when we know such coated cells may exist in circulation We have been fortunate in being able to follow up the blood tests of infants with erythroblastosis for 2 weeks or more We obtained sufficient blood not by venipuncture which is often difficult but by using a Wright tube and collecting from a heel puncture up to 1 to 2 cc of blood which is immediately mixed in the tube with heparin or oxalate By this method we can prove that the mother s antibodies may persist in the child s serum for as long as 10 days or 2 weeks or more which is difficult to understand when we realize that the baby s Rh positive blood is there all the time and should theoretically be taking out or neutralizing the antibody in the serum Some other mechanism is probably operating and it is hoped that some laboratories working on this will be able to disclose why such antibodies are not removed by the Rh positive cells in the baby s blood stream

We persist in trying to substantiate Dr Hill s talk that there might be a third variety of antibodies We have subjected the serums which give a good Coombs test to the same principle and chemical destructive agents as we have used for the antibodies detected by the albumin tests and have as yet found no antibody which would give a negative albumin test and then give a Coombs test that is positive Possibly for this experience we can assume that there is such an antibody or possibly further tests on the part of Dr Hill and his group or Dr Race will confirm our own findings Therefore I wish to commend Dr Chown for his development of this beautifully simple and very efficient and also very saving test This economical test is a very nice way of being able to handle the rarer serums rather than our very wasteful method of using a whole drop of serum and possibly obtaining only 15 to 20 tests from 1 cc

Dr Davidsohn I want to express my appreciation to Dr Hill and the staff and administration of the Baylor Hospital for the privilege of being able to be here at this remarkable meeting I think it is truly remarkable that we consider I think for the first time in history that within about 6 years after a discovery of a new phenomena a congress was called together and arranged so successfully A large number of people interested in one way or another have had an opportunity to hear first hand about the new developments Regarding the question that was brought up by Dr Diamond in his paper as to the origin of the statement that only from 2 to 4 per cent of Rh negative individuals receiving Rh positive blood may become sensitized I too have been interested in this statement and it seems to me the time to track it down This is one of those events which make up a medical mythology The best I could find out was that it was first expressed by analogy to the frequency of fetal erythroblastosis in Rh negative women Then it was simply put in as a fact which need not have any further confirmation I think you ought to be very careful in accepting such medical myths because they cannot be substantiated Dr Diamond s observations are extremely valuable Dr Dameshek s discussion suggested to me that we at

this conference may also take up here in Mexico City the question of terminology that of erythroblastosis versus hemolytic anemia He refers to hemolytic disease hemolytic anemias as being a related subject Suggestions have been made in the literature that erythroblastosis and the terms we apply to erythoblastosis be discarded in favor of hemolytic disease of the newborn and fetus I am not so sure that it is the right thing to do although this term seems to be gaining in popularity I think that it probably is just as bad and just as indefinite as fetal erythroblastosis because at least all of us know what we mean when we say erythroblastosis and the term hemolytic disease of the newborn may be misleading unless we add to it resulting from Rh incompatibility So for the present time it may be desirable to continue to use the term erythroblastosis and to see which term will eventually gain more popularity Dr Potter questioned Dr Race regarding the use of this term by the British workers I reported in the American Journal of Clinical Pathology in 1945 a study in Rh antibodies in which we investigated 3 groups of cases One group where we had only 1 specimen of blood obtain d sometimes long after the baby with erythroblastosis was born And there the incidence of agglutinins was very low Then the third group where the patient was in our own hospital where we could do rep-ated tests and esp-cially tests about 8 to 10 days after the birth of the child we went up to as high as 82 p-r cent Now in this table however you may recall that I had only 62 p-r cent and that is explained in the sam way as Dr Race suggested for British results that we included their low titres of agglutinins which actually we interpret now as being heat stabile agglutinin and therefore th y were excluded from the pre ent list of 62 per cent I agree with Dr Levine regarding th advisibility of investigating the antibodies in th cord blood as a prognostic finding We did it in some cases and we didn t includ it here because we did not have sufficient figures but so far I must say that our impression has not been that there is a striking parallelism between the titre these passively transmitted antibodies and the severity of the diseas in the child or even of the occurence of th disease in the child So I think that we must gather more information of the type of Dr Levine s report Recently wh-n Dr Coombs and his associates first published their paper on the test and then when Dr Hill published his we began using it and we had recourse to old precipitin serums produced years ago by injection of the whole s rum And we found the titre of anti globulin pretty high We got into trouble probably because we at that time were not concerned with the use of only old serum but we used a mixture of all serums I am interested in the report of Dr Race but it seems to me that if one is to produce a good anti globulin serum with albumin then the adsorption of that serum with albumin might also remove th globulin as well as th albumin if that is the homologous antigen used for the immunization

ON CERTAIN VARIATIONS IN ERYTHROBLASTOSIS FETALIS*

By Bruce Chown, M D

DURING the past 2 years my associates and I have investigated 87 families in which 1 or more cases of erythroblastosis due to Rh sensitization have occurred, or in which anti Rh antibodies have been found in the wife s blood but with no discernable disease in the children From these we have selected 60 cases, solely because our data on them are fairly complete The following is a partial analysis of the data

These families came to our notice in 3 ways First, during the past 2 years we have done routine Rh typing on some 15,000 pregnant women During the first year we attempted to follow all Rh negative women so found to termination of the pregnancy The babies were then examined for evidence of erythroblastosis, and when this diagnosis was made the family was further investigated In the past year we have followed only those women in whose blood we found anti Rh antibodies at the time their Rh type was determined All sera were tested against 4 Rh positive and 2 Rh negative cell suspensions in saline against the same set of cells by the albumen technic of Diamond and Denton as modified by Abelson and by the Diamond and Abelson slide technic against fresh O R_1R cells Second we have investigated families referred to us by physicians because of a history suggestive of erythroblastosis Third we have investigated families of children admitted with erythroblastosis to the Children s Hospital of Winnipeg

Statistical results are bound to vary with the method of studying and collecting data No statistical study is valid unless a detailed history is obtained on every family included in the study Without a foreknowledge of the presence of anti Rh antibodies in a woman s blood a number of the babies we have seen would have been passed in the ordinary way as normal Because of our method of collection of data we have not attempted to compare our figures with those of others gath ered in a different way We have included in our 60 families only 3 which we did not interview ourselves and these 3 have been covered by detailed correspondence with the family physicians and with the families themselves We have sought particularly for special modes of sensitization namely transfusion, whole blood injection and abortion Where we have obtained such a history we have placed the families in a separate group as will be seen later for this alters the prognosis

ANALYSIS OF DATA

In tables 1 to 6 are set out the pregnancy results for all 60 families, and a com parison between those families with and those without a history of abortion or transfusion

From the Department of Pathology Children s Hospital and D partment of Pediatrics University of Manitoba Winnipeg Canada

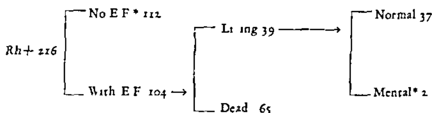
Read at the International Hematology and Rh Conference Dallas Texas Nov 15 1946

* The work on which this report is based was supported by a grant from the Associate Committee on Medical Research of the National Research Council Ottawa Canada I am grateful to the Committee for this support and for permission to publish this report

Of the 17 women in table 3 5 admitted to 1 or more induced premarital abortions 3 had a premarital abortion and Rh positive transfusion 4 a postmarital

TABLE 1 —*Pregnancy Results in All Sixty Families*

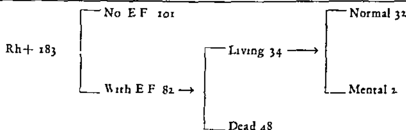
287 pregnancies plus 5 twins = 292 embryos Subtract 15 induced abortions 23 miscarriages 29 known Rh negative and 9 assumed Rh negative total 76 leaving 216 viable Rh positive fetuses



Morbidity 48 1%
Mortality of all Rh+ = 30 1% of those with E F = 62.5%
* In this and succeeding tables E F = erythroblastosis fetalis of any degree Mental = mentally defective

TABLE 2.—*Pregnancy Results in Forty three Families without Abortion or Transfusion*

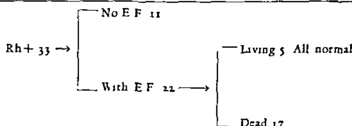
219 pregnancies plus 4 twins = 223 embryos Subtract 13 miscarriages 18 known Rh negative 9 assumed Rh negative total 40 Leaving 183 viable Rh positive fetuses



Morbidity 44 8%
Mortality of all Rh+ = 26 2% of those with E F = 58 5%

TABLE 3 —*Pregnancy Results in Seventeen Families with Abortion or Transfusion All Pregnancies*

68 pregnancies plus 1 twin = 69 embryos Subtract 15 induced abortions 10 miscarriages 11 Rh negative total 36 Leaving 33 viable Rh positive fetuses



Morbidity 66 7%
Mortality of all Rh+ = 51 5% of those with E F = 77 3%

miscarriage and Rh positive transfusion 1 a postmarital Rh positive transfusion for illness 2 had premarital Rh positive transfusions for illness 1 had 2 premarital transfusions for illness but we were unable to trace the donors We have included

1 woman in this group who denied previous pregnancy, transfusion, or blood injection, but who had an anti Rh antibody in her blood at the sixth week of a reputed first pregnancy In our opinion she must have been presensitized in some way

Table 3 gives the results of all postmarital sensitized pregnancies, without reference to the time of sensitization by miscarriage or transfusion The results of pregnancy in these same women subsequent to abortion or transfusion are set out in table 4

TABLE 4 —*Pregnancy Results in Seventeen Families Pregnancies Subsequent to Abortion or Transfusion*

34 pregnancies less 5 miscarriages and 6 Rh negative = 23 viable Rh positive fetuses

Rh+ 23
- No E F 2
- E F 21 →
  - Living 4 all normal
  - Dead 17

Two transfused
Morbidity 91%
Mortality of all Rh+ = 74% of those with E F = 81%

TABLE 5 —*Pregnancy Results after Rh Sensitization Became Manifest Twenty Families without Abortion or Transfusion*

59 pregnancies plus 2 twins = 61 embryos Subtract 5 miscarriages 1 monster dying at birth 5 known Rh negative and 2 assumed Rh negative total 13 Leaving 48 viable Rh positive fetuses

Rh+ 48
- No E F 13
- With E F 35 →
  - Living 8 →
    - Normal 7
    - Mental 1
  - Dead 27

Two possibly three alive due to transfusion
Morbidity 74%
Mortality of all Rh+ = 58% of those with E F = 78%

Abortion and Rh positive transfusion obviously act as sensitizing agents, making it unlikely that subsequent pregnancies will produce normal Rh positive children In families without abortion or transfusion what is the prognosis once sensitization has developed, as evidenced by the birth of an erythroblastotic child? The data on this point are set out in table 5

From tables 4 and 5 it appears that disease is more frequent after a transfusion or abortion than after a first case of erythroblastosis but that the severity of the disease is not definitely greater If however we examine the same data according to the results in each pregnancy as set out in table 6 we get a different impression

The disease if it occurs, appears about equally fatal in the 2 groups but there are no living children after transfusion or abortion, save in the first pregnancy Normal children may occur as late as the fifth pregnancy after the first case of erythroblastosis in the absence of transfusion or abortion With vigorous treatment the child of a sixth pregnancy survived and is normal, all previous 5 children having had the disease and 3 of them dying of it We judge sensitization by transfusion or abortion to be more lethal than 1 and perhaps more, sensitizing pregnancies

TABLE 6 —*Results in Succeeding Rh Positive Pregnancies after Sensitization by Pregnancy Transfusion or Abortion*

	Pregnancy after abort trans or E F													
	0		1		2		3		4		5		6	
	Lived or Died													
	L	D	L	D	L	D	L	D	L	D	L	D	L	D
After first case of E F														
With disease	25	18	3†	9	3‡	4	0	6	1	5	0	3	1§	
Without disease			5		4		2		1		1			
After abortion or trans fusion														
With disease			4*	8	0	4	0	3	0	2				
Without disease			2											

* First pregnancy in which erythroblastosis is believed to have occurred in absence of sensitization by transfusion or abortion Obviously this group cannot include any without disease

† Two treated with intramuscular blood 1 with transfusion

‡ One treated with intramuscular blood 1 with transfusion Latter now mentally defective

§ Replacement transfusion first day Well at 18 months

** Two transfused

ON CERTAIN A B O RELATIONSHIPS

In table 7 are set out the blood group frequencies of 1 000 unselected pregnant women from which population our cases were drawn and the absolute number and frequency of the blood groups of 46 untransfused mothers of erythroblastotic children and of their husbands

A significantly greater number of Group AB women and a significantly smaller number of Group B had erythroblastotic children than one would expect The actual matings were (husband first in each case)

O × O	13	A × O	6	B × O	1
O × A	8	A × A	10	B × A	0
O × B	0	A × B	1	B × B	0
O × AB	1	A × AB	5	B × AB	1

BIRTH OF NORMAL CHILDREN AFTER DISEASED ONES OR AFTER SENSITIZATION BY TRANSFUSION AND SOME FURTHER OBSERVATIONS ON A B O RELATIONSHIPS

In table 6 it is recorded that 13 children without disease were born to mothers who earlier had had 1 or more babies with erythroblastosis and 2 without dis

ease to mothers who had been sensitized by transfusion In most cases we have no positive proof either that those reputed to have erythroblastosis actually had it or that the later born children really were normal We have accepted as probable cases of disease

1 Children dying in the first week with marked jaundice, or in the first 2 weeks with marked anemia

2 Children having a severe jaundice in the first week the jaundice persisting for more than a month with ultimate recovery

3 Children with severe jaundice in the first week with present mental defect, all in families in which the father is Rh positive the mother Rh negative and has anti Rh antibodies in her blood or in one family the father negative the mother positive with anti Hr′ (anti-c) antibody We have usually had to accept the statement of the parents and physician for the normality of later children although we have seen a few ourselves The family records of the 13 children are set out in table 8

The first 9 families seem to us of great importance Admitting the probability that not every case set down as one of erythroblastosis actually was that disease

TABLE [illegible] —*Blood Groups of Parents of Erythroblastotics*

	O		A		B		AB	
General Population	43 4		4 9		9 4		4 3	
	No	%	No	%	No	%	No	%
Mothers of erythroblastotics	20	43 5	18	39 1	1	2 1	7	15 [illegible]
Husbands of these women	22	47 6	2[illegible]	47 6	2	4 8	0	0

and that not every one set down as normal was absolutely normal we are left with a sufficient body of fact to prove that clinically normal children may be born after babies suffering from severe even fatal erythroblastosis In the case of Family Se we have complete proof because we found antibodies in the mother s blood prior to during, and after the last pregnancy and the baby of that pregnancy was entirely normal by every clinical and laboratory test up to 14 days when it went home and has remained normal, according to the mother In the case of Family Ba we treated the 2 children for their erythroblastosis the one between appeared normal to the mother And it is to be remembered that every one of the above women has anti Rh antibodies in her blood today

The fact that normal babies may succeed babies with disease makes it difficult to assess the effect of any treatment given the mother during pregnancy Apparent good results in single cases prove nothing the baby might have been normal anyway On the other hand Nature has in the above families carried out a successful experiment and challenges us to discover how she did it and to devise means to imitate her There must be factors that can modify the Rh antigen antibody hemolytic reaction We have gathered no evidence as to what these may be and do not think it worth while to bother you with our guesses We do suggest that the solution will only come when the major investigation of this

TABLE 8.—*Families in which Normal Children Followed Diseased Children or Were Born Subsequent to Rh Incompatible Transfusion*

No	Name	♂	♀	Pregnancy 1	2	3	4	5	6	7	8	9	10	11	12
				A Without Transfusion											
1	Se	O rr	A R_1R	N	N	IG M A Rr	*N* *A R_1r*								
2	Lu	A R_1	A Rr	N A Rr	Misc	Mole	IG d	*Cong Ht*							
3	Ma	O R_1	A rr	IG d	Ict A Rr	N A R_1r	SB	*EF d* *A R_1r*							
4	Ve	O R_1	A rr	IG d	An d	N O R_1r	Misc	N A P_1r							
5	Ev	O R_1	O rr	N O R_1r	IG d	IG d	N O R_1r	SB	*EF Mac*						
6	Ba	O R_1r	A rr	N Rr	N rr	*IG* *A R_1r*	Prem d	N A R_1r	*IG M* *A Rr*						
7	Ho	O R_1r	O rr	N O R_1r	N O rr	N O R_1r	N O rr	N O Rr	N	IG d	Misc	*N* *O R_1r*			
8	Hu	A RR_1	A rr	N A R_1r	N O R_1r	N	Misc	N	IG d	IG d	N A R_1r	N A R_1r	IG d	N A R_1r	
9	Te	A R_1r	A rr	N	N	N	N A rr	N	N	N A R_1r	N	IG d	N A R_1r	IG A R_1r	N A R_1r
				B Following Transfusion											
10	McM	O R_1r	B rr	N B rr Tr O R_1r	N O rr	N B R_1r	*N* *B rr*								
11	An	B RR	B rr	N B Rr	N B Rr	N B Rr	N B Rr	N O Rr	Misc Tr B RR_1	*N* *B Rr*					

Rh phenotype shown by single symbols e g R_1
Rh genotype by double symbols e g R r
N normal
IG Icterus gravis
M Mental defective
Misc Miscarriage

Cong Ht Congenital heart disease
d died
Ict More than average jaundice in neonatal period cause not determined
SB Stillborn

Mac Macerated
Prem Premature
Tr Transfusion followed by blood group and Rh phenotype of donor
Italics indicate cases seen by us in neonatal period

TABLE 9—*Pregnancy Results in Families in Which Mother or Last Child Was Group B and Mother Had Anti Rh Antibodies in Last Pregnancy*

A Without known transfusion or abortion

Name	♂	♀	Pregnancies							
			1	2	3	4	5	6	7	8
Lu	A R_1	B rr	N O R_1r	Misc	*Ict An* *O* R_1r					
Gr	A R_1R_1	AB rr	N A R_1r	N A R_1r	N AB R_1r	N	N A R_1r	N A R_1r	Misc.	*N* *B* R_1r

B With abortion or transfusion

Name	♂	♀	Transfusions	Pregnancies						
				1	2	3	4	5	6	7
Mo	O R_1	B rr		*N* *B* R_1r						
Ab	O R_1	B rr	Rh+ x 2 Rh−x 1	Mac	Mac	Mac				
Mc	O R r	B rr		N B rr	N O rr	N B R r	*N* *B rr*			
Ad	B R_1R_2	B rr		Ab *	Ab /* Tr B R_1R_2/	*IG* *B* R_1r	Misc			
An	B R_1R'	B rr	B R_1R_2	N B R r	N B R′r	N B R′r	N B R r	N O R_1r	Misc	N *B* R_1r
Dr	A R_1	B rr		Ab	Ab	Ab	Ab	Ab	*Ict An* *O* R_1r	

* Ab = abortion / = bar sinister

Baby Lu was transfused but probably would have recovered spontaneously Baby Dr recovered spontaneously Baby Ad was transfused its jaundice lasted 7 months All the fetuses of Mrs Ab died at about 5 months Mrs Mo had antibodies from 6th week of pregnancy Probably presensitized Abbreviations and italics as in table 8

TABLE 8.—*Families in which Normal Children Followed Diseased Children or Were Born Subsequent to Rh Incompatible Transfusion*

No	Name	♂	♀	Pregnancy 1	2	3	4	5	6	7	8	9	10	11	12
A Without Transfusion															
1	Se	O rr	A R_1R_1	N	N	IG M A R_1r	*N* *A Rr*								
2	Lu	A R_1	A Rr	N A R_1r	Misc	Mole	IG d	*Cong Ht*							
3	Ma	O R_1	A rr	IG d	Ict A R_1r	N A R_1r	SB	*EF d* *A R_1r*							
4	Ve	O R_1	A rr	IG d	An d	N O R_1r	Misc	N A Rr							
5	Ev	O R_1	O rr	N O Rr	IG d	IG d	N O R_1r	SB	*EF Mac*						
6	Ba	O R_1r	A rr	N R_1r	N rr	*IG* *A R_1r*	Prem d	N A R_1r	*IG M* *A Rr*						
7	H	O Rr	O rr	N O R_1r	N O rr	N O R_1r	N O rr	N O Rr	N	IG d	Misc	V O *R_1r*			
8	Hu	A R_1R_2	A rr	N A R_1r	N O R_1r	N	Misc	N	IG d	IG d	N A R_1r	N A R_1r	IG d	N A Rr	
9	Te	A Rr	A rr	N	N	N	N A rr	N	N	N A R_1r	N	IG d	N A Rr	IG A R_1r	N A R_1r
B Following Transfusion															
10	McM	O R_1r	B rr	N B rr Tr O Rr	N O rr	N B R_1r	V *B rr*								
11	An	B R_1R	B rr	N B Rr	N B Rr	N B Rr	N B Rr	N O Rr	Misc Tr B R_1R_2	*N* *B R_1r*					

Rh phenotype shown by single symbols e g R_1
Rh genotype by double symbols e g R_1r
N normal
IG Icterus gravis
M Mental defective
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Cong Ht Congenital heart disease
d died
Ict More than average jaundice in neonatal period cause not determined
S B Stillborn
Mac Macerated
Prem Premature
Tr Transfusion followed by blood group and Rh phenotype of donor
Italics indicate cases seen by us in neonatal period

the last 4 they do not seem to play a part in the first 9 To us this is a group of great importance, suggesting as it does, that other factors than blood incompatibility play an important at times a deciding role in the development or non-development of erythroblastosis

DISCUSSION

Dr Levine Some of these findings certainly take me completely by surprise I hadn't seen cases of that sort I'd seen infants perfectly normal infants following the presence of antibodies in the mother but I've seen the next infant affected The only suggestion I could possibly make is fatigue of the antibody producing cells as he mentioned or perhaps some peculiar conditions of the mother But in one of your cases that would be excluded because the subsequent infant was affected So the affair still remains a mystery What is still more mysterious to me even though the series may be small is the relationship of the blood group of the mother for which at present I have no information whatever I have intended to comment also on the possible relationship of this to Dr Witebsky s findings on the secretors and non secretors but I see Dr Witebsky is still here and perhaps he would like to comment on that

Dr Witebsky The problem of secretion and nonsecretion of Rh soluble substances is a rather involved one We have demonstrated Rh soluble substances in amniotic fluid also in gastric juices and in saliva but the best material to find it in is amniotic fluid and we have been working on that for several years There are several difficulties we are encountering It is difficult to get the material in sufficiently large amounts It is difficult to get the amniotic fluid in amounts we would like to have And I talked about that to Dr Race yesterday Perhaps the difference lies in the sera used One amniotic fluid will inhibit one serum but will not the second or the third The second amniotic fluid will not inhibit the first but maybe the third So it is rather confusing to us at the present time and we are very reluctant to do more about it as far as publications are concerned We concentrated our amniotic fluid by dialysis and have now concentrated the material about 10 times as strong as the original material This material gives us very adequate results and I hope to be able soon to report a little more about it As far as secretion and nonsecretion is concerned there was one antiserum we used which demonstrated that 85 per cent of the people are Rh secretors 15 per cent are non Rh secretors About as similar as in the AB situation but secretion of Rh substances into the amniotic fluid is independent from the AB secretion They are two entirely different things and in 6 or 7 amniotic fluids we obtained from erythroblastotic children we could not find any trace of Rh soluble substances I only report this fact without making any further comments I feel the number is too small but we found 85 per cent of amniotic fluids to have the Rh soluble substance And yet in those 6 or 7 originating from erythroblastotic children we could not find any trace

Dr Davidsohn Dr Chown s paper was interesting Was my impression correct that you found that among mothers the AB group had the higher incidence than the others than one would expect from a normal control series? Now that is interesting because our series of 73 families reported here today when analyzed from the standpoint show such an exceedingly high incidence of AB that I was simply reluctant to include it in this report I was just hoping to get more and to see how much chance played a part but it is interesting that it is in agreement with Dr Chown s report

disease passes from the hands of a serologist or pathologist to those of the internist endocrinologist psychiatrist bio-chemist and obstetrician It will require well organized team work to find the solution to that essential problem how to *prevent* the disease

The fact that the 2 normal children in families 10 and 11 born subsequent to incompatible transfusion, were both Group B and their mothers also Group B, interested us All other families in which the mother or child was Group B and in which anti Rh antibodies were present in the mother s blood in the last pregnancy are set out in table 9

These families do not prove anything We do not know in which pregnancy Mrs Gr first had antibodies whether Mr Dr is homozygous or heterozygous (he is probably heterozygous R_1r) whether Mr Ab is homozygous or heterozygous (he is probably homozygous R_1R_1) what the blood groups were of the macerated fetuses Mrs Dr was receiving endocrine therapy during pregnancy and the baby received some after birth this may have affected the issue In spite of these doubts we suggest that it is worth analyzing more cases to see if blood factor B in any way affects susceptibility to Rh-erythroblastosis The low incidence of Group B women with diseased children suggests this On the other hand Group AB women with the exception of Mrs Gr of table 9 did not fare well Seven without a history of abortion or transfusion had in all 39 probably Rh positive pregnancies Five of the pregnancies ended in miscarriage Of the remaining 34 fetuses 19 died before or after birth a mortality of 56 per cent twice the general mortality of table 2 and equal to that following abortion or transfusion (table 3) Again this suggests the desirability of further study of the blood groups as possible modifying factors in the occurrence of Rh-erythroblastosis

SUMMARY

We have attempted first to show that raw mass statistics on erythroblastosis however valuable they may be in some respects can lead to serious error Results will vary with the mode of collection and with the care with which every family included in the statistics is studied The inclusion of mild often subclinical cases will make the prognosis appear better The inclusion of families in which unrecorded abortions or transfusions have taken place, will make the prognosis appear worse Abortion and transfusion are most active agents in producing disease in later born children

We have further set out our observations on certain blood group relationships and their possible effect on the development of Rh-erythroblastosis These observations we do not regard as more than indications for further study

Finally we have given, so far as we have them the pregnancy summaries of 13 families in which anomalous results occurred namely 9 in which normal children succeeded diseased children 4 in which normal children were born to mothers with anti Rh antibodies in their blood of whom 2 had received Rh incompatible transfusions one had antibodies at the sixth week of a first pregnancy and was therefore probably presensitized and 1 was sensitized by multiple pregnancies only While it is possible that the blood groups may be a factor in

higher degrees of anti A agglutinins than anti B and that in group O mothers with B children the situation was the opposite He suggested the possibility of iso-immunization through the placenta

Dr Levine[9] in 1943 reviewed the subject, establishing that the very suggestive data offered are extensive enough to warrant further investigations

Let us now deal with the evidence which can be obtained on the possibility of isoimmunization against the A and B factors

One of the evidences of the antigenic power of the A and B agglutinogens is the rising titre of agglutinins which is observed following incompatible blood transfusion, for instance A or B type in a type O patient Immediately after blood transfusion the titre is low due to the specific absorption of agglutinins by the incompatible cells This negative phase lasts 2 or 3 days and afterwards the titre is increased several times its orignal level reaching its maximum between 7 and 10 days A second incompatible blood transfusion will produce, in such conditions a stronger reaction, as has been pointed out by Thalheimer[10] and Astrowe [11]

The increase of agglutinin titre following an abortion or the delivery of a stillbirth is another observable fact In these conditions the incidence of abortions or stillbirths in later pregnancies increases This is apparently the cause of the fact observed by Hirszfeld, that the frequency of type A children is less in a mating with A father and O mother in comparison with the cases of O fathers and A mothers the difference being considered due to the loss of type A children by abortions or stillbirths These observations have been confirmed by the statistics of Gardiner and Yerushalmy [1] The antibodies produced by isoimmunization are far more dangerous than the normal antibodies because of their higher titre

Furthermore we have been able to observe that in certain patients who receive repeated blood transfusions there can be abnormal agglutinins as a result of iso immunization in such patients the use of the same donor or of another whose blood contains the same agglutinogen produces a severe hemolytic reaction The same phenomenon can be seen in cases of intramuscular injections of incompatible blood In cases of pregnancy atypical antibodies can be found as a result of iso immunization even in patients who had never received a blood transfusion In such cases the fetus in the uterus is the source of the antigen inherited from the father and when a blood transfusion is needed the cross matching must be done using different bloods and being very careful with the weaker reactions checking them until a completely compatible blood is found

Different observations have been published about incompatibility cases against different blood factors as A_1 O M and P following blood transfusions, and Hill and Haberman[13] have reported a case of sensitization produced by the N factor In some cases the agglutinins found do not correspond to any of the well known blood properties in such cases we can guess that they are due to not very well determined agglutinogens or to multiple sensitizations against several antigens In all cases of isoimmunization one must identify the agglutinin by doing the tests under different temperatures because there are some agglutinins which react better at cold temperatures (anti M A and B) and others react better at 37°C (Rh)

In some cases even if isoimmunization exists it cannot be demonstrated by the

THE A AND B FACTORS AS A POSSIBLE CAUSE OF ERYTHROBLASTOSIS

By Alfonso C Velez Orozco M D

INTRODUCTION

RECENTLY, isoimmunization of the mother by the Rh factor of the fetus and its mechanism have been clearly demonstrated [1] On the other hand, only vague references exist in medical literature regarding sensitization by other blood factors Considering the fact that when red cells pass into the maternal circulation they carry not only the Rh factor but also the blood factors A and B the M N P, Hr and other as yet unknown factors, it is not surprising that immunization by other than the Rh factor may occur

Pregnancy offers a particularly favourable circumstance to produce isoimmunization because of the slow administration of antigen during a lengthy period Therefore this subject must be carefully studied because our lack of knowledge of the causes of fetal death is striking

HISTORICAL DEVELOPMENTS

Dienst [3] in 1905 was apparently the first to think about blood incompatibility as a cause of pathological alterations believing that eclampsia could be produced by the conflicting antigen antibody between the mother and fetus In 1923 Mc Quarrie[4] said that such a mechanism produced not only eclampsia but also the gravidic toxemias and Ottenberg [5] in the same year explained that the passage of blood through the placenta was the mechanism by means of which these phenomena are produced

It was Hirszfeld[6] who in 1928 clarified these ideas creating the term hetero specific pregnancy and applying it to the cases in which the mother and the child had different blood groups In such cases the fetal injury was thought to be caused by an infiltration of agglutinins through the placenta He also thought that the mechanism of protection for the fetus would be the poor sensibility of the erythrocytes to the agglutinins due to the incomplete development of the fetal agglutinogens and the presence of specific group substances in the latter s tissues and secretions Together with Zborowski he presented cases in which the babies weighed less than normally as well as cases in which contrary to what was to be expected according to the laws of heredity the blood type of the mother was more frequent in the babies than that of the father This was thought to be due to the fact that in the already mentioned incompatibilities sterility or at least a diminution in fertility was present

Since then much research has been directed to determine the antigenic capacity of the A and B factors Biancalana and Teneff[7] in 1930 were the first to prove the antigenicity of the A and B agglutinogens in human species Jonsson[8] in 1936 found that mothers belonging to the group O who had group A children developed

From the Institute of Medical and Biological Research Mexico City Mexico
Read at the International Hematology and Rh Conference Dallas Texas Nov 15 1946

We must also take into consideration that a placental defect which permits the passing of the fetal blood is necessary although it should not be expected that a considerable lesion of the placenta must exist to produce isoimmunization Dr Levine[15] has demonstrated that a small amount of fetal blood acting during a long period of time is sufficient to produce an effective degree of isoimmunization and Dr González Guzmán[16] insists upon the fact that we should take into account the destruction of fetal cells during its normal processes of development, because the stroma of such cells contain the antigens and in such conditions the passage of cells through the placenta is not necessary, but the products of the lysis of the cells can yield a sufficient antigenic stimulus

On the other hand there are constitutional fetal factors which must be taken into account principally their characteristic of being secretors or nonsecretors These substances widely studied by Witebsky [17] can be demonstrated as present in fetal tissues after the sixth month As an important detail we will only point out that there are haptens consisting of 2 factors one soluble in alcohol and of lipoid origin and the other one soluble in water These substances may be sought for in saliva, because if they do not exist there they cannot be found in any other secretion

According to our unpublished data, 82 per cent of our population are secretors In the remaining 18 per cent nonsecretors the A and B factors are limited to the erythrocytes

The small number of cases we have studied seems to confirm our previous supposition that when the fetus belongs to the nonsecretor group it presents a more or less typical picture of erythroblastosis and when it is secretor the blood group substances in the tissues cause a more generalized deleterious effect of the agglutinins producing early abortions or stillbirths We have not been able to determine the secretor or nonsecretor character of feti or stillbirths, having deduced such characteristics through genetic studies of its possible heredity These statistical data will be published later

Concerning this isoimmunization we have seen in addition to typical erythroblastosis subclinical anemia of the newborn repetitive abortions or death of the fetus in the uterus Therefore it can be said as a general rule that these troubles are not a perfectly defined unity such as fetal erythroblastosis or hemolytic disease of the newborn because we consider that 2 conditions must be fulfilled in order to speak about typical erythroblastosis first the presence in peripheral blood of erythroblasts with the typical alterations pointed out by Dr I Gonzalez Guzman and second the confirmation of sensitization by any test preferably the developing test using the Race Mourant and Coombs serum

The presence of erythroblasts in the peripheral blood means a response to destruction of red blood cells from the bone marrow But it is important to distinguish erythroblastemia from erythroblastosis In the first case we only have the presence of normal erythroblasts in the blood stream and it can be taken as a slight alteration of the haematopoiesis produced by anoxemia, while in the second case the structural changes signify a profound attack on the reticulo-endothelial system which produces erythrocytes

usual tests due to the fact that the technics are not very sensitive or because the patient is in the negative phase Sometimes we must admit that we are dealing with antibodies present in the tissue cells having in such cases the biologic test as the only resource

In other instances incompatibility can produce hemolysis instead of agglutination in the tests made in vitro This must always be taken into account to avoid mis interpreting the results

About the isoimmunization in pregnancy we can state the following the agglutinogens have been demonstrated by Kempt[11] in the blood of a fetus of 37 days This can be explained if we remember that the first red blood cells are formed in the yolk sac in the fourth week of pregnancy having been found constantly after the second or third month The agglutinins appear later, and when a child presents them at birth it can be considered, according to Hirszfeld and to our personal observations, that they are maternal, having passed through the placenta These

TABLE 1 —*Pregnancies or Marriages*

Compatible			Incompatible		
Husbands or Fetus		Wife or Mother	Husband or Fetus		Wife or Mother
O	×	O	A	×	O
O	×	A	B	×	O
O	×	B	A	×	B
A	×	A	B	×	A
B	×	B	AB	×	O
O	×	AB	AB	×	A
A	×	AB	AB	×	B
B	×	AB			
AB	×	AB			

agglutinins disappear in approximately 10 days and the proper agglutinins are then originated

In Rh cases the first pregnancy almost always gives normal children even though they be Rh positive due to the fact that a sufficient degree of isoimmunization has not been developed We have seen in several cases that when a blood type incompatibility is added the disease is presented in the first pregnancies Many examples have been observed of Rh negative type O mothers with Rh positive type A husbands who in spite of never having had a blood transfusion had a stillbirth in the first delivery

When the frequencies of the blood types of a certain population are known the frequency of the mother fetus combination can be mathematically established We see that this kind of sensitization is not so common as could be expected if we consider the percentage of incompatible marriages Attempts to explain such a difference by several mechanisms have been tried

In the first place we must consider the incapacity of some mothers to produce antibodies as can also be seen in Rh cases There are some people who because of certain genetical or constitutional characteristics are anergic to certain allergens

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A brief historical review is presented, establishing some evidence for the antigenic capacity of the A and B blood factors, such as the experimental production of serum in animals, the increase of agglutinin titre following an incompatible transfusion or intramuscular blood injection and heterospecific pregnancies Some fetal antigens are analyzed, as well as the possible pathogenic process of sensitization Some factors of the antigen antibody conflict in the fetal organism are studied The pregnancies are also analyzed from the viewpoint of the fetal maternal incompatibility explaining the low frequency of the observed cases, due to nonpermeable placentas the anergy of the mother, and the fetal characteristic of being secretor or nonsecretor, considering that the clinical form of the fetal alteration depends upon the presence or absence of A and B substances in fetal tissues The writer gives as a basis to consider a true isoimmunization the presence in the blood stream of erythroblasts with nuclear alterations and a positive developing test

A clinical case of twins in which the sick twin was of incompatible blood type in respect to the mother is presented

ACKNOWLEDGMENT

I am in debt to Miss Graciela Romero for her assistance

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In most of the cases of A and B sensitization, hemolysis is not found in spite of the increased titre of agglutinins normally present It has been explained by some investigators as due to the existence of blood substances in the tissue cells and in the parenteral liquids and secretions of the fetus We shall now study a very demonstrative clinical case which we have chosen among 13 cases seen by us

TABLE 2

	Blood Type	Secretor	Other Factors
Mother	B	S	MN Rh''_0
Father	A_1	s	N Rh_0
First twin (sick)	A_1	S	MN Rh_0
Second twin (normal)	B	S	MN Rh''_0*

* The Rh subtypes were only determined recently because before we had only the 85% serum

REPORT OF CASE

E R a 42 year old woman had had 3 normal children with her first husband with the second she had female twins The serological characteristics are studied in table 2

The 3 children of the first marriage were B Rh positive

When the first twin a week old was seen by us she presented anemia of 3 200 000 R B C and had been jaundiced since the fourth day of life In her peripheral blood 27 erythroblasts for 100 leucocytes were found presenting the same characteristics as in erythroblastotic cases She received four 50 cc blood transfusions of A Rh positive blood perfectly tolerated and recovered completely

The mother had never had blood transfusions or abortions A week after delivery she presented a titre of 1 8192 against A_1 blood the titre being actually 1 256

Even though she was Rh $_0$ the existence of atypical agglutinins mainly anti Hr was carefully investigated They as well as tests for syphilis were negative

DISCUSSION

This is a case of isoimmunization due to the A factor The A agglutinogen is not a simple substance but comprises 2 properties called A_1 and A_2 by Landsteiner and Levine Each one presents its own agglutinins The proportion of A is 25 per cent in reference to A_1 Some investigators think that they are the same antigen varying quantitatively If this is true it will explain why we have only seen sensitization against A_1 fraction which seems to be the most antigenic

This case is of great interest to us because it has its own control the sick twin is the one who has the opposite blood type to the mother Also we can see the clear increase of titre because the normal ones for Mexico according to our small number of determinations are $\frac{1}{32}$ to $\frac{1}{128}$ with extreme values from $\frac{1}{4}$ to $\frac{1}{512}$

We think according to the above mentioned case that we have demonstrated that the A and B blood factors can in certain occasions and conditioned by circumstances which we do not as yet know be the cause of isoimmunization which can manifest itself by erythroblastosis repetitive abortions or fetal death

SUMMARY

The author establishes that there is no reason to think that the isoimmunization must be limited to the Rh factor especially in pregnancy cases because such a condition brings the ideal means for its presence

filled with needle like brownish masses or fine brown granules of bile pigment[12] and biliary stasis ensues Fatty degeneration is noted and in some areas the liver cells are loaded with fat (figure 1) [13 14] Other liver functions become depressed There is hypoproteinemia with attendant loss of circulating fluid Anoxia becomes more pronounced and the functions of the liver become more deficient Death ensues in such cases usually in 1-3 days Prevention of anemia only partially mitigates against this cycle The most prominent initiating cause is the hemolysis and this is not prevented by simple repeated transfusions

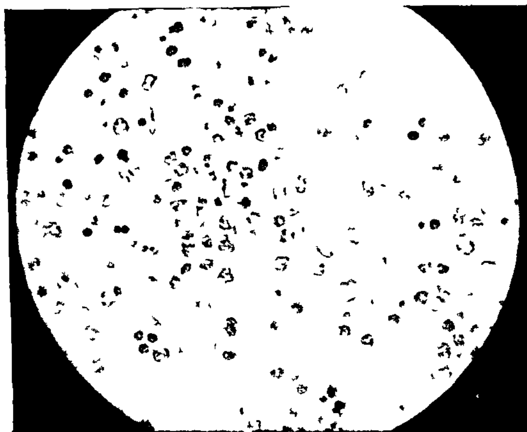


FIG 1a LIVER—MARKED EXTRAMEDULLARY ERYTHROPOIESIS AND FAT DROPLETS IN HEPATIC CELLS

The liver changes described above have been repeatedly reported in the earlier literature [13–17] Klemperer[14] suggested that therapy be directed at conserving liver function This was attempted by Hart [18] who asked a surgeon to perform a substitution on a baby with icterus gravis in 1925 before the liver cells have been too extensively damaged The disregard shown in the subsequent literature to Hart s experiment is no more astounding than the fact that Mitchell[19] in 1928 proved the basic hemolytic origin of erythroblastosis fetalis by demonstrating a specific hemolytic antibody not associated with normal isoagglutinins in the serum of mothers of jaundiced babies This antibody was found in the serum of 51 per cent of the mothers of jaundiced babies and in many of the cord bloods as well and specifically hemolyzed the cells of the infants in vitro When the role of

THE TREATMENT OF ERYTHROBLASTOSIS FETALIS BY SUBSTITUTION TRANSFUSION

By HARRY WALLERSTEIN, M D

THE treatment of erythroblastosis fetalis by the substitution of Rh negative *blood for the baby s original Rh positive erythrocytes in a simultaneous ex* sanguination transfusion procedure was suggested[1] because

1 Other methods of tranfusion therapy proved only partially effective Even before the theory of isoimmunization of the mother by an antigen in the fetal red blood cells was postulated by Levine [3 4] and the antigen identified as similar to one experimentally produced by Landsteiner and Wiener,[5] blood transfusion was the treatment of choice Blood was given by subcutaneous intramuscular or intra peritoneal injection but the repeated intravenous administration was considered best [6] Although it was noted that results following transfusion were somewhat better if nonfamily donors were used the father usually acted as the source of blood It is now known that father s blood is entirely unsuitable because it always carries the same antigen as the blood of the affected infant This was also true in 85 per cent of the cases in which professional donors were chosen at random Many of the infants appeared to suffer the equivalent of a transfusion reaction with increasing jaundice toxicity and fever and 80 per cent of these infants died irre spective of the form of therapy [7] When Rh negative blood was used to replace the infant s hemolyzed erythrocytes the untoward reactions did not occur and anemia was swiftly brought under control Nevertheless from 35-50 per cent of these infants still died [8] Autopsy usually showed findings identical to those previously reported even though anemia and transfusion reactions were no longer factors The reasons are obvious when one recalls that all or nearly all of the baby s Rh positive blood would frequently be destroyed [9] and the infant actually became temporarily Rh negative to the test [10]

2 Postmortem examination of the infants who died despite repeated transfusion with Rh negative blood revealed that death was usually due to overwhelming toxicity *induced by liver insufficiency It is probable that kernicterus occurs only* after such liver damage [11] The role of the liver in the pathogenesis of erythroblas tosis has been schematically shown by Davidsohn[1] and his description clearly illustrated the reasons why former transfusion methods were so often ineffective The destruction of the Rh positive erythrocytes is the initiating agent The liver cells partially compressed by dilated sinusoids and by centers of extramedullary hemopoiesis are called upon to excrete the products of hemolysis If the excretory function of the hepatic cells is not impaired it is probable that even large amounts of bilirubin can be excreted [11] In a baby with excessive blood destruction and con sequent anoxia this function can readily depreciate The bile canaliculi become

From the Erythroblastosis Fetalis Clinic Jewish Memorial Hospital New York City New York Read before the International Hematology and Rh Conference Mexico City Mexico November 21 1946

isoimmunization in the pathogenesis of erythroblastosis was proven attention was focussed upon the resulting anemia, and it was not till simple transfusion therapy proved ineffective that the part played by the liver was restressed by Davidsohn Substitution transfusion[1] was then applied in an attempt to prevent the irreversible liver changes A search of the literature subsequently disclosed the work of Hart two decades earlier

The purpose of the substitution procedure is to remove the baby s Rh positive erythrocytes without subjecting it to the perils of shock and to provide it with circulating and functioning red blood cells which will survive Three methods to accomplish this aim have thus far been devised



Fig 2 Sagittal Sinus Technic
Rh positive blood withdrawn through anterior fontanelle as Rh negative blood is simultaneously administered through median cephalic vein Note comparative size of needle in insert

I THE SAGITTAL SINUS ROUTE

The substitution transfusion using the sagittal sinus as the route for bleeding (figure 2) is similar to the method employed for Hart

Technic

1 The infant is immobilized

2 A superficial vein is exposed a cannula inserted and an infusion of isotonic sodium chloride or compatible human plasma is given by gravity

3 A ¾ inch 19 gage needle is inserted into the longitudinal sinus through the anterior fontanelle and withdrawal is performed by syringe

4 The first and last specimens removed are saved for differential agglutination tests The other specimens are discarded but a count is kept of the amount of blood withdrawn

Fig 1b Liver—Note the Inspissated Bile Collections Within the Liver Cells

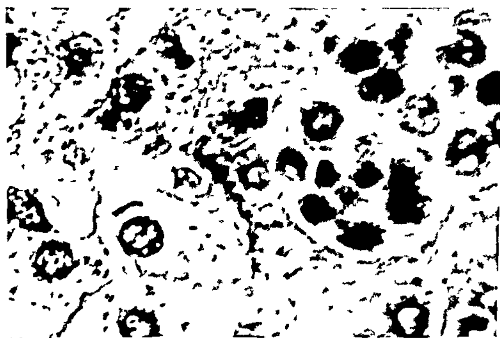


Fig 1c Liver—Biliary Canaliculus Completely Outlined and Filled with Inspissated Bile

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5 After 50 cc of blood have been withdrawn compatibl Rh n gative blood replaces the infusion

6 When an estimated substitution of about 85 p r cent has been accomplished the needle is removed from the sinus The estimate is bas d upon the infant s weight figuring the blood volume as 10 per cent If 1 blood volume has been removed and replaced a substitution of 63 per cent has been achieved If 2 volumes have been exchanged a substitution of 86 per cent has been accomplished

7 The usual procedure is to remove 500 cc of blood replacing with 575 cc of Rh negative blood

8 During the procedure 10 cc of 10 per cent calcium gluconate are injected by syringe through the cannula in the vein This tends to counteract th effects of the large amount of citrate in th infused blood[20] as well as to replace some of the calcium removed in the bleeding process

Since this method requires the insertion of a needle into the sagittal sinus some pediatricians have expressed fear of possible brain injury It is agreed that care must be employed with this or any therapeutic method but in our experience the use of a short needle and the limiting of the use of the sinus to blood withdrawal definitely preclude the chance of subdural hemorrhage or lasting trauma to the adjacent brain tissue

II THE RADIAL ARTERY ROUTE

The radial artery (figure 3) was first used for this purpose by Polayes [1] in an attempt to find an alternative method The heparinization of the infant was subsequently suggested by Vogel[22] who experienced difficulty in bleeding from the artery

Technic

1 The infusion is set up in the usual way

2 The infant s hand is extended at the wrist and the radial artery exposed

3 The artery is cannulated and the blood permitted to drip into test tubes or oth r receptacles for testing and discard A count is maintained of th amount of blood removed

4 Occasionally the vessel is too small to admit a cannula It may th n be ni ked and permitted to bleed dir ctly into the test tubes If bleeding slows gentle rubbing of the vessel with a gauz sponge will remove th clot and reinitiate free flow of blood

5 When the substitution has b en accomplished the artery may either be ligated or bl ding controlled by a pressure bandag

6 Calcium gluconate is used in th usual way

Some objections can also be raised to thic technic Anomalies in this region are uncommon and there is usually a good anastomosis with the interosseous and ulnar arteries to maintain the circulation in the hand However if such an anomaly did exist there could be some impairment of circulation This can be prevented by the simple precaution of tying the artery before it is cut to see if there is any change in color or temperature of the hand If there is no apparent change the artery may be used for bleeding If there is a change in the hand one of the other routes for bleeding should be cho en

Objection has been raised to the use of heparin in this procedure We have found this drug unnecessary and have experienced no greater difficulty without it than with it In view of Potter s findings [3] that silent intracranial injury is common in the newborn it would appear that heparin is not only unnecessary but risky as well Wiener [4] states that heparinization is necessary to obtain free bleeding and that it is not dangerous since it is washed out of the circulation in the process of substitution



FIG 3 RADIAL ARTERY TECHNIC
Cannula in radial artery with bleeding into collecting test tube

III THE UMBILICAL VEIN ROUTE

Recently a third method of substitution has been devised (figure 4) The umbilical vein has long been used for transfusion immediately at birth [5 6] but Diamond [7] has suggested that the vessel may be used for alternate bleeding and

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The radial artery (figure 3) was first used for this purpose by Polayes [1] in an attempt to find an alternative method The heparinization of the infant was subsequently suggested by Vogel who experienced difficulty in bleeding from the artery

Technic

1 The infusion is set up in the usual way
2 The infant s hand is extended at the wrist and the radial artery exposed
3 The art ry is cannulated and the blood permitted to drip into test tub s or oth r receptacles for testing and discard A count is maintained of th amount of blood removed
4 Occasionally the vessel is too small to admit a cannula It may then be nicked and permitted to bleed dir ctly into the test tubes If bleeding slows gentle rubbing of the vessel with a gauz sponge will remove th clot and reinitiate free flow of blood
5 When the substitution has been accomplished the artery may either be ligated or bl ding con troll d by a pressure bandag
6 Calcium gluconate is used in th usual way

Some objections can also be raised to thic technic Anomalies in this region are uncommon and there is usually a good anastomosis with the interosseous and ulnar arteries to maintain the circulation in the hand However if such an anomaly did exist there could be some impairment of circulation This can be prevented by the simple precaution of tying the artery before it is cut to see if there is any change in color or temperature of the hand If there is no apparent change the artery may be used for bleeding If there is a change in the hand one of the other routes for bleeding should be cho en

Objection has been raised to the use of heparin in this procedure We have found this drug *unnecessary and have experienced no greater difficulty without it than* with it In view of Potter s findings [3] that silent intracranial injury is common in the newborn it would appear that heparin is not only unnecessary but risky as well Wiener [1] states that heparinization is necessary to obtain free bleeding and that it is not dangerous since it is washed out of the circulation in the process of substitution

in the newborn, and therefore extra precautions are needed Antibiotic therapy is given routinely after this method is used and occasional idiosyncrasies may be encountered The possibility of dislodging a partially adherent thrombus with subsequent embolization must also be kept in mind, although no such event has yet been reported

In consideration of the respective merits of the 3 routes here described, it is believed that none is completely without risk yet any one may be used with safety if care is exercised It is suggested that the pediatrician or hematologist who may be called upon to perform substitutions attempt to familiarize himself with all 3 methods, so that if there is difficulty with one route an alternative technic will be possible

It appears wise to prohibit breast feeding in all of these cases It has been shown[28] that the maternal antibody can appear in the milk and it is assumed that if these substances are absorbed unchanged in the newborn digestive tract they may add to the hemolysis

Occasionally an infant whose blood has been substituted may show a secondary drop in hemoglobin 6–8 weeks later This is due to the gradual exhaustion of the transfused erythrocytes and the retarded resumption of full blood formation by the bone marrow A single transfusion, even of Rh positive blood, will prove corrective at this time

INDICATIONS FOR TREATMENT

We do not believe that substitution should be performed as a routine treatment for erythroblastosis fetalis Certain criteria have been established which are presumptive of the imminence of severe erythroblastosis either in late pregnancy or in the early neonatal period

1 *Before Birth*

A *History of Previous Erythroblastosis* Multiparity is an important predisposing factor This is the most important single fact in predetermining the fate of the current pregnancy Potter[29] states that erythroblastosis is ten times more frequent in multiparous than in primiparous women Unless the father is heterozygous the illness will reappear with increasing severity in each successive pregnancy

B *Serologic Studies* The mere demonstration of Rh incompatibility in a family is not sufficient indication of impending disease in the fetus or newborn It is especially important to demonstrate antibodies in the mother s serum during the pregnancy [30] These too have been found without subsequent illness in the offspring [2, 31] If the tests are repeated at regular intervals and the concentration of antibody rises steadily it may be assumed that the agglutinin is not residual from a previous immunization but is related to the current pregnancy This should alert the obstetrician to the possibility of erythroblastosis so that he may be prepared to have a substitution performed if it is proved necessary

C *Additional Criteria* (1) A history of repeated stillbirths or late miscarriages [32, 33] (2) Possible previous immunization of the mother by transfusion [34] (3) Increase in mother s uric acid and icterus index [35] (4) Excessive enlargement of the uterus [35] (5) Edema of the fetal scalp as visualized by x ray [36]

2 *At Birth*

A *Physical Examination* Icteric amniotic fluid large pale placenta golden yellow vernix caseosa splenomegaly and jaundice or anemia in a baby whose mother gives a typical history or serologic picture may be taken as evidence of actual disease and indication for immediate treatment

B *Laboratory Findings* The demonstration of maternal antibody in the umbilical cord blood and the

replacement or for bleeding while the transfusion is given through a superficial vein We have found this technic effective and no more difficult than the other methods The use of the umbilical vein has shown that it and the ductus venosus remain open at least anatomically for considerably longer than formerly supposed We have performed the substitution by this technic as late as 36 hours after birth with no appreciable increase in technical difficulty With this method too heparin has been suggested Again we have found it unnecessary and believe it to be risky

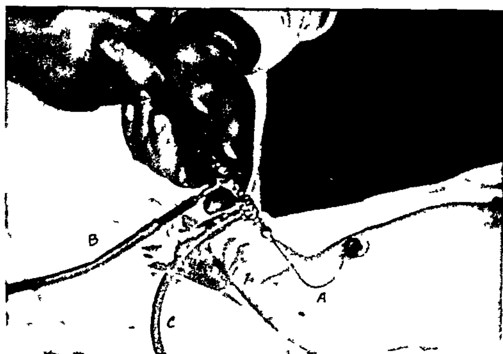


Fig 4 Umbilical Vein Technic
A Catheter in umbilical vein B Tube to infusion bottle C Tube to discard bottle

Technic

1 The umbilical cord is cut at the tie and the vein located If there is a thrombus in the vein it is removed

2 A pliable plastic catheter with a bore just large enough to permit the passage of a 19 gage needle is introduced into the vein Occasionally there is some resistance at the skin level but with a minimum of manipulation the catheter will pass easily through the umbilical vein and ductus venosus into the inferior vena cava

3 When the catheter reaches the vena cava a show of blood appears at the outlet

4 A 19 gage needle is fitted snugly into the outlet of the catheter and attached to 2 three way valves in series To each valve a length of rubber tubing is attached one leading to the discard bottle the other to the infusion bottle

5 Fifty cc of blood are withdrawn by syringe and then equal amounts alternatingly removed and replaced until the desired substitution has been accomplished

6 Calcium gluconate is then injected through the catheter which is then removed and the cord is retied

We have experienced no complications with this method One must remember however that infection of the umbilical vein is one of the common causes of sepsis

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sensitization of the cord erythrocytes with blocking antibody are indications of impending and actual hemolysis These do not however indicate the ability of the infant to excrete the products of such hemolysis This function can better be demonstrated by changes in the icterus index and the number of nucleated red blood cells In the absence of direct evidence of illness we repeat these tests every 2 hours and treat the infant only if the icterus index rises and the number of nucleated red cells fail to drop or if clinical evidence of erythroblastosis begins to app ar If th se indications do not app-ar within the first 24 hours substitution will not be needed and the infant will recover with exp-ctant treatment

The Rh incompatibility is responsible for erythroblastosis in 92 per cent of the cases The remaining cases result from immunization against the A or B isoagglutin ogens [37] against the Hr factor[38] or against subgroups of the Rh factor [39] Treat ment is the same except that the blood should be chosen on the basis of com patibility with the mother s serum Blood of the same group as the infant is preferred but if not available group O cells suspended in compatible plasma may be used

CONCLUSIONS

The basic pathology of erythroblastosis fetalis necessitating treatment by blood substitution is described The role of the liver in the pathogenesis of the illness is illustrated

The technics of 3 methods of blood substitution are described and illustrated The indications for therapy are enumerated

Twenty seven infants have been treated by substitution transfusion Six of these died but 4 of the 6 were not treated until past the 24 hour period and until jaundice and toxicity were marked Of the remaining 2 1 had a tentorial laceration with massive hemorrhage as an obstetric accident Among the 23 survivors there have been no sequelae The oldest infant was born in May 1945

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tests capillary blood, occasionally a finger puncture that is not deep enough that doesn t seem to produce a free flow of blood will allow clotting of the drop of blood a little more rapidly on the slide and thereby make the reading of a positive test unreliable or a negative test unreliable If capillary blood is to be used, it is important to use either a deep fingerprick or a prick of the lobe of the ear with a sharp needle and not squeeze too much or use another stab if necessary to get a free flow of blood

There is one further question I would have in the record, and that is on the question of *bone marrow hypoplasia or exhaustion in an erythroblastotic infant who has exhibited huge hemolysis* That I think we noted and described more than 15 years ago in the original series of 20 cases of hemolytic anemia in the newborn or erythroblastosis Bone marrow hypoplasia is almost a uniform complication of any severe hemolytic anemia in the newborn After the second week or third week of life has passed, even with multiple transfusions the infant often goes into a relatively aplastic state Sometimes this is associated with actual hypoplasia of the marrow evidenced by marrow puncture and aspiration Quite often it is merely an instance of functional hypoplasia in that the marrow looks quite hyperplastic by aspiration but the reticulocytes remain very low, for a period of 2 3 or up to even 6 weeks but the infant may not recover his marrow function efficiency until after the second month of life It is important to realize this and we feel that we should not overtransfuse the child during this time unless the child exhibits symptoms of blood want like diarrhea vomiting failure to gain or poor appetite Usually this relative hypoplasia is attended by an otherwise good state of health Transfusion should be resorted to if the infant begins to show other symptoms or if the blood level falls below 2½ million erythrocytes or to 50 per cent hemoglobin Otherwise it is not necessary after the second or third week to keep the child s blood level up to 5 million by frequent transfusions and there is some suggestion in statistical evaluation of such cases that too frequent transfusion may prolong the period of relative hypoplasia

Dr Levine Here is another for Dr Diamond or Dr Levine I think I will give it to Dr Diamond

Dr Diamond QUESTION *What is the possible fate of the Rh antibody after hemolysis in vivo has occurred? Can it be liberated and reused to destroy more blood? If not, how do you explain the long continued hemolysis occurring in some erythroblastotic infants?*

Dr Diamond ANSWER These are all rather difficult questions because they are the subject of a lot of controversy and have been for some time I don t believe anyone has the answer to the problem as yet I shall be glad to hear any corrections or suggestions from any other members of this gathering There is evidence that the Rh antibody at least in vitro can be detached from the red cells by either heating and vigorous shaking or by other mechanical means I think Dr Levine can tell us about that That was one of Landsteiner s technics wasn t it for separating antibodies from antigen even after the combination had taken place But we have no evidence in vivo such antibody is neutralized in the usual cases that show prolonged hemolysis for 3 or 4 weeks One occasionally sees a baby in whose serum one can demonstrate the continued existance of Rh antibodies usually of the so called

CURRENT PROBLEMS REGARDING THE Rh FACTOR

AFTER LUNCHEON DISCUSSION, NOVEMBER 15 1946 INTERNATIONAL HEMATOLOGY AND Rh CONFERENCE DALLAS TEXAS MEXICO CITY, MEXICO

Dr Hill I would like to introduce everyone at the table Most of them need no introduction but we will run down the line Dr Dameshek editor of the Journal of Blood and Professor of Medicine Tufts Medical School, is at my right and next to him is Dr W H Strother, Chief of our Obstetrical Service at Baylor Next is Dr Bruce Chown of Winnipeg, Canada next is Dr Race of London next is Dr Louis K Diamond of Harvard, next is Dr Alfonso Velez Orozco of Mexico City Secretary of the Mexican Transfusion Congress, next is Dr Witebsky whom you heard this morning Dr Philip Levine Mrs Levine Dr Uribe Guerola, President of the Mexican Transfusion Congress and on my left Dr Davidsohn of Chicago Dr Henry Winans Chief of our Medical Service at Baylor Hospital, Dr Gonzalez Guzman of Mexico City whom you heard this morning and Dr Wong from far away China Dr John Scudder of New York and Dr Sol Haberman of Baylor and Secretary of this conference

I have asked Dr Levine because of his position in this field, if he would serve as chairman and moderator to assign these questions as he sees fit and to help out in every way possible to get these questions answered So I will call on Dr Levine to come to the microphone and handle these questions, and he will assign them to those speakers to whom they refer

Dr Levine QUESTION *Is bovine albumin available and if so where?* I ll ask Dr Diamond to anwswer that

Dr Diamond Bovine albumin can be manufactured far cheaper than human albumin and in stable form consistently Bovine albumin is now being manufac tured by the Armour Company of Chicago Illinois We should be careful to ask for albumin for Rh testing purposes since the salt content should not be neglected if uniformly successful results are to be obtained It is put out in 30 per cent solution It can be diluted to 20 per cent which is correct for anti Rh testing but it should not be diluted beyond 15 per cent for it tends to be ineffective below that per centage

The albumin should be diluted with saline solution because if one dilutes it with water the salt content may become too low and there again the reaction won t be entirely satisfactory I don t know the exact cost at the moment some of you probably know that better than I do but I think it is in the neighborhood of two dollars for 20 cc of about 30 per cent solution

QUESTION *Medical Technologist Mr Galaway wants to know if it is true that capillary blood is less reliable than venous blood for Rh testing*

Dr Diamond Possibly Dr Chown can answer part of that He uses not only capillary blood but venous blood by a capillary tube method as you all know and thereby, out comes the Scotch in him because instead of 20–30 tests from 1 cc of anti Rh serum which is too wasteful he probably gets 60–100 or more We find no difference in capillary blood and venous blood the only difficulty is that if one

throblastosis with the mother's own breast milk. Maybe somebody else's breast milk.

Dr Levine Thank you, Dr Witebsky. We have only about a half hour for all the other questions, so I will have to ask everyone to be brief and concise. There are questions in Spanish so I will ask Dr Uribe to read them and translate.

Dr Uribe QUESTION *Is there a possibility of neutralizing the Rh antibody?*

Dr Levine ANSWER Neutralization of the Rh antibody analagous to the neutralization of the AB substances is not possible in vivo. In test tubes we can, of course, absorb the anti Rh agglutinins by means of the proper Rh positive cells. However as Dr Witebsky has found, the Rh substance is present in small quantity in the amniotic fluid. Possibly some isolated antigen may be hoped for. If such a substance could be isolated in sufficient quantity, as has been done with the A and B substances, one might then hope for in vivo neutralization of the Rh antibody in the immunized Rh negative person.

QUESTION A Doctor from Mexico directs this question to Dr R R Race. *Have you applied anti E, anti e, anti d or anti c to cases of disputed paternity and if so what is the status of such evidence in court?*

Dr Race ANSWER In answering this question I should point out that we have none of the anti-d serum available to us and therefore haven't used it. We have not applied these sera to such legal procedures in that the British government appointed a commission to decide whether paternity tests should be compulsory in all cases. I believe they reported very favorably on having such a policy, but during this time the war was started and the subject has been forgotten. I am sure that in the near future this question will be revived and an official decision will be reached.*

Dr Levine There are a few questions directed to me and I will attempt to dispose of them rapidly.

QUESTION *Can you give some explanation for the possible use of typhoid vaccine in an attempt to prevent the formation of anti Rh antibodies?* This is asked by Dr Marcia.

ANSWER This suggestion has been made in the literature as a possible method of diverting the reticulo-endothelial system to the production of antibodies other than the Rh. I do not feel at all in accord with such a statement in that such injections may well serve to stimulate further antibody response rather than to act as a diversion. An alternate approach might be that of blocking the reticulo-endothelial cells as has been done through the use of finely divided carbon which acts to prevent any antibody formation as has been done in the past in experimental animals. However, I would not like to start such experiments in humans because of the obvious accompanying dangers. It would be far better to perform such experiments first in experimental animals. Ideally we would want to have a substance, preferably the hapten of the Rh positive cell, which could then be used directly in the neutralization of antibodies in the pregnant mother.

QUESTION *Miss Massey wants to know how you explain the presence of a blocking antibody in a nonsensitized individual.*

* These tests have been successfully applied in cases of disputed paternity in the United States and evidence regarding them has been admitted in court. *Eds*

blocking or incomplete or hyper immune variety for 1 or 2 weeks or longer We are usually reassured that the baby will recover rapidly and not have too prolonged an anemia if within the first few days the maternal antibodies are no longer detectable in the child s serum I can t offhand recall any cases which showed pro longed hemolysis after such antibodies had been used up or had disappeared from the baby s serum in the second or third day I d be very glad for a report on the elaboration of the results in the interim

Dr Levine The antibody can be separated from the cell but whether it occurs in vivo is questionable If it can be separated this is more than I know Laboratories have succeeded in separating all the blocking antibodies they have to use ex ceptionally low temperatures or in certain cases they had to use heat You get of course destruction of the red cell and I doubt whether it is split off At least there is no evidence Dr Diamond I m sorry we have another question for you Would you come in Dr Diamond

QUESTION *Why does an Rh incompatibility resulting in transfusion reaction in adults give evidence of intravascular hemolysis hemoglobinuria while one never sees intra vascular hemolysis in an erythroblastic infant?*

Dr Diamond ANSWER I don t know that I d agree that one never sees intra vascular hemolysis or hemoglobinemia in the infant with erythroblastosis Cer tainly one sees a high degree of jaundice and within the first 24 hours we have often been able to find actual hemoglobinemia in the serum It may have something to do with the metabolism of the tissues but the speed with which they are con verted may cause the case to differ in the symptoms

Dr Levine I have 2 questions addressed to Dr Witebsky *1 Dr Scudder wants to know why Eli Lilly has given up the manufacture of the AB substance where can it be be purchased and does it meet with your specifications?*

Dr Witebsky I don t know why Eli Lilly has given up the production of AB substance They produced 60 000 vials and they felt it wasn t necessary for a while but Sharp & Dohme have taken up the production of AB substance I get the im pression that while they are doing a good job they are not quite ready yet for the general distribution but I believe they have quite a good preparation Specifications have been drawn up at the National Research Council and the manufacturer is expected to follow those specifications Of course the preparation of the A and B substances is a relatively complicated chemical procedure and cannot be done in everybody s laboratory We feel that some manufacturers should do it

Dr Levine Dr Ross asks Dr Witebsky *What is the present opinion with reference to the desirability of breast feeding of an erythroblastotic infant?*

Dr Witebsky Rh antibodies are present in the mother s milk especially during the first few days and especially in the colostrum Colostrum is almost as rich in Rh antibodies as serum After a week or two the Rh antibody concentration drops down However we feel these children should not be fed breast milk because the antibodies can be transferred to the newborn child we and believe that the new born child can abosrb these and that antibodies usually reach the blood via this site But that holds true only for the first few weeks of birth After that that phenomenon stops occurring But I would not feed a child suffering from ery

throblastosis with the mother's own breast milk Maybe somebody else's breast milk

Dr Levine Thank you, Dr Wiener We have only about a half hour for a the other questions so I will have to ask everyone to be brief and concise There are questions in Spanish so I will ask Dr Uribe to read them and translate

Dr Uribe QUESTION *Is there a possibility of neutralizing the Rh antibody?*

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* These tests have been successfully applied in cases of disputed paternity in the United States and evidence regarding them has been admitted in court *Eds*

ANSWER That is not possible Anti Rh antibodies whether agglutinins or blocking antibodies cannot be found in a normal Rh negative individual who has not had a stimulus Now it is possible that the Rh substance may have a wide dis tribution, say in some of our food products and that idea has been mentioned in connection with the origin of the anti A and anti B antibodies but so far there is no evidence that that s correct

QUESTION *Is there any difference in results from the use of saline suspension of red cells and of citrated whole blood in the capillary test for Rh?*

Dr Choun We do our tests with the capillary tube under two conditions First of all the commonest one we do is a fast blood examination where the blood is sent in in a test tube In that case we always take off the cells and wash them once in saline and suspend them in saline but we can also do it directly from the finger using citrate and we never have any trouble with it under those circumstances You can use either citrate or saline but routinely we use saline washed cells *

Dr Levine Dr Scherer asks *Can the blocking antibody be used successfully in the typ ing of blood for transfusions?*

Dr Chown ANSWER The blocking antibody can be used in the slide test as suggested by Dr Diamond or in the test tube when serum or albumin is used for the suspension medium It is preferable of course to use the saline agglutinin for such work

Dr Levine What explanation do you offer for the fact that some symptoms of erythro-blastosis especially jaundice sometimes do not appear for a number of hours after birth? Would Dr Diamond care to answer this question?

Dr Diamond At present I can offer no explanation as to why such conditions are delayed in their appearance We have found cases in which high titre antibodies were found in the mother and the child fails to show symptoms for 2 or 3 days Sometimes in such cases the anemia will not appear for as much as 6 days after birth while however the jaundice may appear relatively early even before definite symptoms of active hemolysis is evident So many variables enter into the pro-duction of the various symptoms and laboratory findings that it is difficult to pre dict the exact condition that will appear in the child The question as to whether the child s hematopoietic system can rebuild cells rapidly enough is one we need to evaluate considering each of these factors in trying to explain the symptoms I believe Dr Chown will tell you in his paper tomorrow of cases of hemolytic anemia which did not appear even though high titre antibodies against the infant s red cells were found

Dr Levine Dr Edith Potter asks me whether it is safe to transfuse with Rh positive blood when the antibodies have disappeared from the baby s serum

Dr Levine ANSWER I think it is pretty safe to do that but in practice I would still recommend continuing with Rh negative blood I would do that merely on the basis that what we detect in the test tube is actually a crude determination of the antibody, and does not give you the real picture of what is happening in vivo

* Washing of R B C is not necessary if saline diluted serum is used—that is serum of sufficient potency to permit 1 4 or better dilution *Eds*

However, in theory one may assume that when the antibody disappears from the child s blood stream, it is safe to administer Rh positive blood One can further check the results by watching for a rise in the hemoglobin and red cell count

QUESTION *Is it possible to use the Rh negative blood of the mother to transfuse the Rh positive baby and how should it be prepared?*

Dr Levine ANSWER The whole blood of the mother is never to be used for transfusions to an erythroblastotic child This can be easily understood in that the etiology of the disease is due to the antibodies which may be found in the mother s blood Consequently a transfusion of whole blood from the mother would add further destructive elements to the child s circulation However if neither a blood bank nor a suitable Rh negative donor is available I suppose one can remove the plasma from the mother s blood, wash the cells, and substitute normal saline and then give a transfusion with these cells Actually we should never have to resort to the use of the maternal cells

QUESTION *In routine typing for Rh what objection is there to suspending the individual cells in their own serum?*

Dr Levine ANSWER There is no real objection to such a procedure By the use of this method one can use blocking antibodies for the typing of human erythrocytes Of course, it is preferable to use the agglutinin for routine typing

QUESTION *What is the longest period of time the Rh antibodies have been found to persist once isoimmunization has occurred?* Would Dr Chown care to answer this?

Dr Chown The longest period of sensitization I have observed is a case in which 35 years elapsed between the last antigenic stimulus and the detection of these persistent antibodies As far as I know there were no intervening antigenic stimulations in this case

Dr Levine I believe it is safe to assume where women are concerned, that the antibodies may not last as long as such exceptional cases However, it is good to remember that to all intents and purposes these women are permanently immunized and all further transfusions even after long periods of time have elapsed should be done only with Rh negative blood

QUESTION Dr Andujar directs these questions to Dr Velez *What is the incidence of Rh negative blood in Mexican Indians? Does this finding re enforce the theory that the Mayan civilization is Mongolian in origin, and that the Aztec and Toltec are Asiatic?*

Dr Velez There will be a paper presented later in this meeting by Dr Salazar Mallen about the incidence of the Rh type among Mexican Indians It is our belief that the American Indians came from migratory Asiatic peoples however the blood group studies do not bear this out We know that the B blood type is not common among our Indians while B is very common among the Asiatic peoples Dr Salazar Mallen will probably discuss this problem when he presents his paper In my own work an Rh negative pure Indian is an extremely rare finding I have yet to find one such case The results of such tests I believe are still inconclusive and much more work needs to be done before a conclusion can be reached

Dr Levine This question is directed to Dr Dan Campbell by Dr Haberman *How can you explain or what theoretical explanation can be offered for the differences between the saline agglutinin the blocking and third order antibody phenomenon?*

ANSWER That is not possible Anti Rh antibodies whether agglutinins or blocking antibodies cannot be found in a normal Rh negative individual who has not had a stimulus Now it is possible that the Rh substance may have a wide distribution, say in some of our food products and that idea has been mentioned in connection with the origin of the anti A and anti B antibodies but so far there is no evidence that that s correct

QUESTION *Is there any difference in results from the use of saline suspension of red cells and of citrated whole blood in the capillary test for Rh?*

Dr Chown We do our tests with the capillary tube under two conditions First of all, the commonest one we do is a fast blood examination where the blood is sent in in a test tube In that case we always take off the cells and wash them once in saline and suspend them in saline but we can also do it directly from the finger using citrate and we never have any trouble with it under those circumstances You can use either citrate or saline but routinely we use saline washed cells *

Dr Levine Dr Scherer asks *Can the blocking antibody be used successfully in the typing of blood for transfusions?*

Dr Chown ANSWER The blocking antibody can be used in the slide test as suggested by Dr Diamond or in the test tube when serum or albumin is used for the suspension medium It is preferable of course to use the saline agglutinin for such work

Dr Levine What explanation do you offer for the fact that some symptoms of erythroblastosis especially jaundice sometimes do not appear for a number of hours after birth? Would Dr Diamond care to answer this question?

Dr Diamond At present I can offer no explanation as to why such conditions are delayed in their appearance We have found cases in which high titre antibodies were found in the mother and the child fails to show symptoms for 2 or 3 days Sometimes in such cases the anemia will not appear for as much as 6 days after birth while however the jaundice may appear relatively early even before definite symptoms of active hemolysis is evident So many variables enter into the production of the various symptoms and laboratory findings that it is difficult to predict the exact condition that will appear in the child The question as to whether the child s hematopoietic system can rebuild cells rapidly enough is one we need to evaluate considering each of these factors in trying to explain the symptoms I believe Dr Chown will tell you in his paper tomorrow of cases of hemolytic anemia which did not appear even though high titre antibodies against the infant s red cells were found

Dr Levine Dr Edith Potter asks me whether it is safe to transfuse with Rh positive blood when the antibodies have disappeared from the baby s serum

Dr Levine ANSWER I think it is pretty safe to do that but in practice I would still recommend continuing with Rh negative blood I would do that merely on the basis that what we detect in the test tube is actually a crude determination of the antibody and does not give you the real picture of what is happening in vivo

* Washing of R B C is not necessary if saline diluted serum is used—that is serum of sufficient potency to permit 1 4 or better dilution *Eds*

However, in theory one may assume that when the antibody disappears from the child s blood stream, it is safe to administer Rh positive blood One can further check the results by watching for a rise in the hemoglobin and red cell count

QUESTION *Is it possible to use the Rh negative blood of the mother to transfuse the Rh positive baby and how should it be prepared?*

Dr Levine ANSWER The whole blood of the mother is never to be used for transfusions to an erythroblastotic child This can be easily understood in that the etiology of the disease is due to the antibodies which may be found in the mother s blood Consequently a transfusion of whole blood from the mother would add further destructive elements to the child s circulation However if neither a blood bank nor a suitable Rh negative donor is available I suppose one can remove the plasma from the mother s blood, wash the cells, and substitute normal saline and then give a transfusion with these cells Actually we should never have to resort to the use of the maternal cells

QUESTION *In routine typing for Rh what objection is there to suspending the individual cells in their own serum?*

Dr Levine ANSWER There is no real objection to such a procedure By the use of this method one can use blocking antibodies for the typing of human erythrocytes Of course, it is preferable to use the agglutinin for routine typing

QUESTION *What is the longest period of time the Rh antibodies have been found to persist once isoimmunization has occurred?* Would Dr Chown care to answer this?

Dr Chown The longest period of sensitization I have observed is a case in which 35 years elapsed between the last antigenic stimulus and the detection of these persistent antibodies As far as I know there were no intervening antigenic stimulations in this case

Dr Levine I believe it is safe to assume, where women are concerned that the antibodies may not last as long as such exceptional cases However it is good to remember that to all intents and purposes these women are permanently immunized and all further transfusions even after long periods of time have elapsed should be done only with Rh negative blood

QUESTION Dr Andujar directs these questions to Dr Velez *What is the incidence of Rh negative blood in Mexican Indians? Does this finding reenforce the theory that the Mayan civilization is Mongolian in origin, and that the Aztec and Toltec are Asiatic?*

Dr Velez There will be a paper presented later in this meeting by Dr Salazar Mallen about the incidence of the Rh type among Mexican Indians It is our belief that the American Indians came from migratory Asiatic peoples, however the blood group studies do not bear this out We know that the B blood type is not common among our Indians while B is very common among the Asiatic peoples Dr Salazar Mallen will probably discuss this problem when he presents his paper In my own work an Rh negative pure Indian is an extremely rare finding I have yet to find one such case The results of such tests I believe are still inconclusive and much more work needs to be done before a conclusion can be reached

Dr Levine This question is directed to Dr Dan Campbell by Dr Haberman *How can you explain or what theoretical explanation can be offered for the differences between the saline agglutinin, the blocking and third order antibody phenomenon?*

Dr Campbell This is somewhat similar to the question I wanted to ask Dr Haberman I m like most of you I guess I ve thought a lot about this problem but as yet there is no clear evidence as to what the blocking antibody may be I think immuno-chemists are beginning to change their opinions of antibodies and now have decided that the problem should be re examined in the light of this newer knowledge Antibodies are so heterogeneous that very seldom do we detect any thing but the good reacting antibodies which fortunately the rabbit usually pro duces We do find now in some cases rabbits will produce anti sera which are extremely heterogeneous and this heterogeneity is due to a variety of things such as the variations in valence of the antibody molecules A different case, of course would be where antibody molecules have only 1 combining site other molecules having 2 or 3 and the ones which are usually detected in serological reactions are those which have 2 or 3 combining sites Besides this question of valence we have all sorts of heterogeneity due to the strength of combining sites of antigens with antibodies and so it is within these classes of antibodies which are very difficult to detect by the usual serological reactions I think we shall have to place the so called blocking antibody in this category In the case of adding serum to bring about the serological reaction the result might be due to the fact that the univalent antibody might conflict with the high concentration of protein which has many combining sites and therefore completes the serological reaction There are many cases of blocking anti sera we have been working with recently in which the pro teins can be aggregated by a variety of methods such as anti coupling reagents or denaturation reagents which will cause complex aggregates to take on the property of agglutinating cells

Dr Levine We will request that this discussion continue following the paper by Drs Hill and Haberman

HISTORICAL REVIEW OF MEXICAN BLOOD TRANSFUSION

BANQUET ADDRESS BY DR EDUARDO URIBE GUEROLA, INTERNATIONAL HEMATOLOGY AND Rh CONFERENCE DALLAS TEXAS—MEXICO CITY MEXICO November, 1946

President Dr Hill Medical Authorities of Dallas, Texas Dear Doctors Ladies and Gentlemen

This conference takes place in affiliation with the Second Mexican Blood Transfusion Congress

Now allow me to state briefly the outstanding points of Mexican Medical History Mexico is a name which, in the minds of many persons, has had connotations quite different from reality For some people it appears to be exotic, for others it is romantic and for yet others it is an odd country What I want to say briefly is that the first inhabitants of Mexico the Aztecs, Mixtecas, Toltecas, Otomies Chichimecas and Mayas practiced medicine according to their times, and the Goddess Tzapotlaltenan presided over general medicine Blood was named eztli They noticed the circulation of blood by the beating at the end of the heart called tetecuicaliztli and by the radial beating to which they attached the name tlahuatl y tetecuicoca However for them, medicine was considered a trade Training was given as that of a trade and knowledge was handed down from father to son with the fathers teaching their sons the correct names of the diseases and how to cure them by means of herbs Surgical instruments were kept and used in suitable cases

Ancient Mexicans obtained their medicines from the three kingdoms of nature animal, mineral, and vegetable Of the three kingdoms the vegetable kingdom was the one to which they paid the most attention and the one which they came to know best Taking advantage of their knowledge has made it easier to study the several species of plants forming groups to be used according to modern therapeutics

As you well know Christopher Columbus discovered the American continent on the twelfth of October 1492 Later in the year 1519 Hernando Cortes arrived in the city of Mexico From that date to September 1821 when Mexican independence was consummated Mexican soil was a Spanish colony There are four transcendental facts related to that period of time which are necessary to point out Mexico was vested with the privilege to possess before any other country on the American continent the first hospital, the first printing press, the first university,* and the first book of medicine The first hospital on the American continent was founded in 1524 It was given the name Hospital de la Limpia Concepcion and finally the name Hospital de Jesus a name which it has up to the present time This hospital has been giving uninterrupted service for a period of 422 years It is a building of large courtyards and handsome arches part of which is considered the most ancient construction that the city possesses On the other hand the remaining

*This statement might be challenged both in Peru and in the Dominican Republic *Eds*

part is devoted to a most modern hospital having all up to date improvements in hospital technic

The first printing press established on the American continent arrived in Mexico in 1539

The first university on the American Continent was founded in 1553 Its name was Real Universidad de Mexico It is worthwhile to mention the curious fact that the public ceremony announcing its inauguration took place in the church of the school of Sn Pablo de los Agustinos This church rebuilt in 1570 and transformed in 1932 is today the auditorium of Juarez Hospital and, in this auditorium will take place one of the meetings of this Second Mexican Blood Transfusion Congress

In Mexico in 1570, was printed the first book on medicine that was published on the American continent entitled Opera Medicinales by Francisco Bravo

During the seventeenth century medicine progressed Great progress in surgery was made during the eighteenth century On the tenth of April 1770 the Real College of Surgery was inaugurated

During the third and last period from Mexico s independence up to the present time we find that once Mexican independence was consummated in September of 1821, the twenty third of October 1833 the Mexican Faculty of Medicine was founded It is today 113 years old and since 1854 has occupied the same building This building was called the Inquisition because in it was held the terrible In quisition Tribunal from 1736 until 1820

The Mexican Medicine Faculty during the nineteenth century progressed in general form emerging from the metaphysical period into the positive relying on prominent directors and professors At present in the year 1946 the attendance of students in the Faculty of Medicine is as high as 4 500

Two hospitals cooperate fundamentally with the Faculty for the teaching of clinic and surgery the General and the Juarez hospitals

The General Hospital had a predecessor the Sn Andres Hospital, founded in 1779 which was replaced by the present General Hospital inaugurated in 1905 This hospital has always excelled as the leader of medicine in Mexico

The Juarez Hospital the place where we shall meet next week has been con sidered from its founding up to the present time as the Mexican School of Surgery It was founded as a hospital in 1847 Next year it will complete 100 years of con- tinuous service The building which it now occupies had its origin in the sixteenth century Fray Pedro de Gante founded the Parish of Indios de Sn Pablo which was under the jurisdiction of the Franciscan priests In 1575 the Augustin religious order took charge of the church and established there the Augustins school A short time later part of the building was used as a military headquarters Later it was transformed into a hospital On August 23 1847 it was inaugurated with the name of Hospital de Sn Pablo Since that time it has been considered the munic ipal hospital of the city It was under the charge of the Sisters of Charity and changed its name to Hospital Juarez in 1872 a name which it keeps up to the present time During the nineteenth century and for a part of the twentieth century the Hospital Juarez had undergone several improvements in construction in an attempt to modernize it For those who do us the honor of entering this old building we take the liberty to remind you that the house is 100 years old and does not rely on

modern installations Our dear hospital is old fashioned, modest, but, nevertheless, it is still the leading surgery school in Mexico The Juarez Hospital Surgical Society was founded in 1930 I have the honor of being the President of this institution at the present time Its motto is *to better surgery through constant collaboration* In order to obtain and give general knowledge in surgery, in 1932, the Surgery Society founded the Institute of National Surgeons Assemblies Six assemblies have taken place, one every two years We are now celebrating the seventh one and we consider it an extraordinary privilege that this International Conference takes place in affiliation with the Seventh Assembly

Honored gentlemen with this brief summary I have tried to give you an idea of the history of medicine in the country where we are going to have the pleasure to have you as our guests We are extending to you a most cordial welcome and are asking in a most kindly manner to make yourselves at home

In the aspect of blood transfusions, it is convenient to point out that Mexican historians have found facts that give them assurance that the first blood transfusion performed on the American continent took place in the City of Mexico in 1845 in a case of hemmorrhage a fact that has been jotted down in several publications at that time and that has been scrupulously examined and analyzed by the historian, Dr Jose Alcantara The first publication on blood transfusion appeared in Mexico in December of 1874 under the title Studies of Blood Transfusion This study is of particular interest since it expresses the ideas of that time and since some of them are still accepted today

Interest in blood transfusions mounted steadily until 1925 In 1930 numerous services were established and the work of publication and teaching was evident By this means it was made possible for the Republic to enjoy its benefits The uarez Blood Transfusion Center under my care has contributed in an important measure to the progress and knowledge of transfusion This center was founded in 1932 and has published thirty six papers In 1937 the center was represented at the Second International Blood Transfusion Congress which took place in Paris France and at which so many important facts were decided with regard to blood groups preserved blood hematological and organization problems On the twenty-fourth of April 1938 through the initiative of the Juarez Hospital the Mexican Blood Transfusion Society was founded In May 1938, it gave a postgraduate course In November 1941 it realized a great accomplishment the friendship of the William Buchanan Blood Plasma and Serum Center and of its Director Dr Joseph M Hill From the beginning this friendship has been a solid one and has made it possible that Baylor and Juarez work together with the best understanding I render my deepest homage to Dr Hill for his knowledge and extraordinary work, a demonstration of which are the brilliant results of these conferences and the success which they have obtained through world acknowledgement and recognition Nor can we fail to recognize that all this has been made possible by the exceptional organization obtained by Chairman Dr Hill and his secretary Dr Haberman, to whom I direct my enthusiastic congratulations

Then in August of 1942 in the Juarez Hospital was founded the first blood bank in the Republic In November 1942 the first Mexican Blood Transfusion Congress

was established Its purpose was to obtain a general knowledge of transfusion problems with special emphasis upon blood groups and the usage of plasma And we have arrived at important conclusions Now, in November 1946 we are celebrating the Second Mexican Blood Transfusion Congress and this International Conference Baylor and Juarez wish to work together toward general orientation in the second of the greatest discoveries relating to transfusion in the present century, i e the discovery of the Rh factor We intend to arrive at conclusions particularly on those points

1 Which technics should we recommend for the routine investigation of the Rh factor and its subtypes?

2 What nomenclature of the Rh and its subtypes should be adopted?

3 Is it not incumbent on us to explore the possibility of establishing an International Hematology Association possibly with biannual meetings, taking into consideration that air transportation brings all countries close together not only in space but in ideas as well Such an organization could promote our scientific advancement

We want to hear your opinions in order that we may consider them from now until our last meeting on Saturday November 23, in Mexico City so that it will be possible to arrive at conclusions concerning these points which we have submitted to you

Baylor and Juarez wish to thank with all our compliments the official speakers of the Conference and of the Congress for their very valuable cooperation We do not forget that they left their very important occupations and came to give us of their knowledge We recognize too the presence of each one of the attendants which contributes and gives more significance to the meetings and we beg you to join with us in expressing our gratitude for the great contributions to human welfare and scientific advancement made by the late Dr Karl Landsteiner

We are doing our best to do good work We are making our best effort to obtain a good result And we *shall* do it regardless of the truth about knowledge which is so beautifully expressed in the words of the famous English poet

THOU knowest all I seek in vain
What lands to till or sow with seed
The land is black with briar and weed
Nor cares for falling tears or rain

THOU knowest all I sit and wait
With blinded eyes and hands that fail
Till the last lifting of the veil
And the first opening of the gate

THOU knowest all I cannot see
I trust I shall not live in vain
I know that we shall meet again
In some divine eternity

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